THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

FOUNDED BY JOHN J. ABEL

OFFICIAL PUBLICATION
OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND
EXPERIMENTAL THERAPEUTICS INCORPORATED

VOLUME 89
1947

BALTIMORE, MARYLAND

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JOHNSON REPRINT CORPORATION
111 Fifth Avenue, New York 3, New York

Johnson Reprint Company Limited
Berkeley Square House, London, W. 1.

First reprinting, 1963, Johnson Reprint Corporation

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ERRATA

VOLUME 88, NUMBER 4, DECEMBER 1946

Page 373: Change first equation to read:

$$H + Fe\ (\bar{C}\bar{N})^{-}_{6} \longrightarrow H^{+} + Fe\ (\bar{C}\bar{N})^{-}_{6}$$

Page 374, Paragraph 1, line 6: Change "digitoxin" to "digitonin" and read "Merck digitonin was used"

Page 381: Insert in last line of text the words "of the sex" and read "to a lesser degree in the case of the sex hormones."

TESTS OF 2,3-DIMERCAPTOPROPANOL AND RELATED DITHIOLS FOR DECONTAMINATION OF LEWISITE ON HUMAN SKIN[1]

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Received for publication June 12, 1946

The evaluation of decontaminants of human skin after application of arsenical vesicants was an important task of this laboratory during 1942 and 1943. Before the advent of 2,3-dimercaptopropanol (BAL) (1), we had demonstrated that oxidizing agents had decidedly limited decontaminative utility; apparently they could react only with that fraction of the vesicant which was not combined with the skin, and were unable to reverse the damage produced by the arsenicals through inhibition of enzyme systems dependent upon sulfhydryl groups for activity (2).

In June 1942, samples of BAL were made available to us for testing on human skin. We obtained excellent results in prevention of vesication by lewisite, with BAL either in an ointment vehicle or diluted in glycol-type solvents (propylene glycol, dioxane, "Cellosolve", etc.). Pure BAL was considered too toxic to apply undiluted to human skin.

It was soon discovered that BAL had some limitations. Even at short intervals after contamination, a few men would develop blisters after treatment, particularly in hot weather. Furthermore, BAL is fairly toxic, and produced dermatitis on a few of the men tested.

Therefore, forty-three compounds related to or derived from BAL were tested in this laboratory for decontaminative efficacy. Sixteen of these were water-soluble derivatives, some of which could be prepared as crystalline solids. In addition, twenty-eight other sulfur compounds such as monothiols, thiourea derivatives, and thioacids were examined. Over 5000 men were used in this program.

METHODS. *Application of the vesicant.* In the earlier tests carried out in this laboratory, two sizes of stainless steel rods, with areas at the tips of 1.23 and 1.69 sq.mm., were used for the delivery of lewisite to the skin; the smaller one delivered about 50 μg. and the larger one about 90 μg. The lesions produced on a group of men were uniform and generally reproducible.

In most of the tests reported here, the vesicant was applied by a micropipette which

[1] Under the direction of Dr. William Bloom, these tests were carried out as a joint activity of the Committee on the Treatment of Gas Casualties of the Committee on Medical Research and the University of Chicago Toxicity Laboratory (an establishment of the National Defense Research Committee).

The facilities for the tests requiring human volunteers were furnished by the Senior Medical Officer of the Ninth Naval District. Most of the men used in these tests were volunteers from the Recruit Training Command, Great Lakes Naval Training Center, Great Lakes, Illinois. Lt. Comdr. A. F. Abt, Lt. Comdr. T. B. Friedman, and Lt. J. H. Heinen, all of the Medical Corps, U.S.N.R., were assigned by the Navy Department to participate in the program.

We acknowledge with pleasure the cooperation and sympathetic understanding we have received, in this as well as our other vesicant testing activities, from Capt. E. W. Brown (MC) U.S.N., of the Bureau of Medicine and Surgery, U. S. Navy.

delivers multiples of 0.05 cu. mm. (95 μg. of lewisite) with a high degree of consistency and precision (3).

Decontamination. The procedure for testing new compounds as decontaminants of lewisite on human skin was usually the following: Redistilled lewisite was applied by rod or micropipette to symmetrical sites on the flexor surfaces of both forearms of a group of men (usually 15 or 20 per group). No previous treatment, such as washing, was given the arms. After 40 minutes, the right arm was treated with the new dithiol, 0.4 M. in propylene glycol, applied by a Band-aid saturated with 0.25 cc. of solution.[2] The left arm was either treated with a similar solution of BAL for direct comparison of the new compound with BAL, or left untreated in those experiments in which only the qualitative efficacy of the compound was of interest.[3]

Readings. The men's arms were inspected 48 hours after application of the vesicant, and the occurrence and size of both erythemas and blisters were noted. No other attempt was made to grade the severity of the lesions. It was not necessary to use the reduction in size of erythemas and blisters as a criterion of decontaminative efficiency, since the reduction in frequency was usually more significant.

Evaluation. When the new and the standard decontaminants were compared on opposite arms of the same group of men, a difference in number or size of blisters or erythemas usually was noted. When the difference was not clear-cut, the procedure was usually repeated with a longer interval between contamination and treament. At a 60 minute interval, there was in most of our tests a considerable difference in decontaminative efficacy of the various compounds.

When two compounds were tested on different groups of men, again it was usually easy to decide whether or not one compound was superior to the other. There were instances, however, when the minor differences observed were of doubtful significance; in these instances, the compounds in question were compared directly.

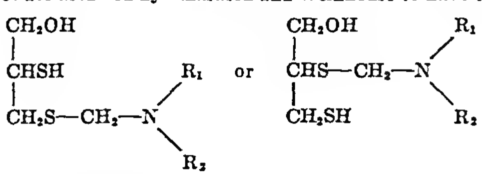
MATERIALS. Several preparations of BAL were used in these experiments; most of them appeared identical in decontaminative activity. The American Reference standard BAL (1) was used in most of the comparisons of BAL with other dithiols.

The simpler derivatives of BAL were supplied by Dr. W. A. Lazier and co-workers at the DuPont Experimental Station (NDR code). The structures of these compounds are evident from their names listed in Table VIII.

The majority of the water-soluble derivatives were prepared by Dr. M. S. Kharasch and Dr. A. Weinhouse of the Department of Chemistry of the University of Chicago (KD code); a few were later submitted by Dr. Lazier.

Most of these compounds were prepared from water-soluble amines by condensing the amine with formaldehyde, and then reacting the azomethine with BAL (4). Some of the derivatives which were prepared from sodium salts of amino acids could be prepared as crystalline solids by precipitation from acetone. Usually, the starting materials were mixed, and the solution diluted to the desired concentration and then tested without actual isolation of the product.

These derivatives are assumed by Kharasch and Weinhouse to have the structure:

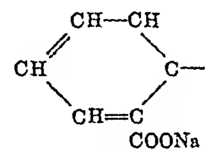
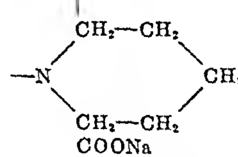


The former structure is considered more likely.

[2] In some tests in which ointments were used, 0.05 cc. of the ointment was extruded from a syringe and rubbed in over a 500 sq. mm. area.

[3] Although comparisons of decontaminants tested on different groups of men are less reliable than comparisons of those tested on opposite arms of the same men, untreated controls are of considerable importance in cold weather, when the small doses of lewisite used in these tests will fail to produce blisters on an appreciable number of men.

The following compounds of this type have been tested:

CODE NO.	NAME	R_1	R_2
KD-2, NDR 287	Diethanolaminomethyl thioether of BAL	$HOCH_2CH_2$—	$HOCH_2CH_2$—
KD-7	N-*o*-Carboxyphenyl-N-methylaminomethyl thioether of BAL, sodium salt	CH_3—	
KD-8	N-Carboxymethylaminomethyl thioether of BAL, sodium salt	H—	$NaOOCCH_2$—
KD-9	N-Carboxymethyl-N-methylaminomethyl thioether of BAL, sodium salt	CH_3—	$NaOOCCH_2$—
KD-10	N-phenyl-N-sulfomethylaminomethyl thioether of BAL, sodium salt	C_6H_5—	$NaOSO_2CH_2$—
KD-11, NDR 288	N-methyl-N-glucitylaminomethyl thioether of BAL	CH_3—	$CH_2OH(CHOH)_4CH_2$—
KD-15	2-Carboxypentamethyleneaminomethyl thioether of BAL, sodium salt		
KD-37	N-Carboxymethyl-N-2-hydroxyethylaminomethyl thioether of BAL, sodium salt	$HOCH_2CH_2$—	$NaOOCCH_2$—
KD-38	N-Carboxymethyl-N-acetylaminomethyl thioether of BAL, sodium salt	CH_3CO—	$NaOOCCH_2$—
KD-39	N-Carboxymethyl-N-benzoylaminomethyl thioether of BAL, sodium salt	C_6H_5CO—	$NaOOCCH_2$—
NDR 475	Acetamidomethyl thioether of BAL	H	CH_3CO—

Similar compounds have been prepared in which both mercapto groups were bound. These were ineffective as decontaminants of lewisite.

Other water-soluble derivatives which were tested were salts of BAL: ethylene diamine salt (KD-43), diethanolamine salt (KD-44, NDR 289), and the glucitylmethylamine salt (NDR 290).

EXPERIMENTAL WORK. *Tests of BAL against Lewisite: Compilation of results of all tests.* A large number of tests of BAL against liquid lewisite were carried out under widely varying conditions of dose of lewisite, concentration of BAL, interval between contamination and treatment, temperature, and relative humidity.

A general summary of the results is presented in Table I, a compilation of all of the tests in which BAL in an eye ointment[4] or in propylene glycol was used against liquid lewisite. Included for the purpose of comparison are the data obtained with peroxides and peracids. The difference between BAL and the peroxides is striking: the former protected 73% of the men from both erythema and blisters, while the latter completely protected only 38%; the difference in

TABLE I

Comparison of BAL and peroxides as decontaminants of Lewisite on human skin (all tests)

Dose of Lewisite: *ca.* 50 μg. to 400 μg. Interval Lewisite to treatment: 2′ to 80′.

DECONTAMINANT	NO. OF MEN	ERYTHEMAS		BLISTERS	
		No.	%	No.	%
Peroxides and peracids (3% to 15% H_2O_2)	2449	1525	62%	1042	43%
BAL (0.08 M to 0.8 M)	1821	498	27%	308	17%
Untreated lewisite (controls)	4671	4568	98%	4331	92%

prevention of vesication alone is of comparable magnitude. It should be mentioned that variations in temperature, dose of vesicant, and time interval between application of lewisite and treatment were equally distributed between the two types of treatment.

BAL was found to be effective against other arsenical vesicants such as phenyldichloroarsine, ethyldichloroarsine, etc., as well as lewisite. In addition, a mercury vesicant, 2-thienyl-mercuric chloride, can be successfully neutralized by BAL.

Effect of amount of Lewisite used. The decontamination of 50 μg. of lewisite was more nearly complete than the decontamination of 90 μg. of lewisite by 0.05 ml. of 0.4 M. BAL in ointment applied 60 minutes after contamination; only two blisters and six erythemas developed after treatment of the 20 sites receiving 50 μg., in contrast to the 6 blisters and 16 erythemas occurring after treatment of 20 sites receiving 90 μg. All of the untreated skin areas in both groups developed blisters. At shorter time intervals, the effect of varying the amount of lewisite

[4] Formula: Peanut oil 10%, lanolin 10%, cetyl alcohol 5%, glycerol monosterate 8%, benzyl benzoate 5%, liquid petrolatum 62%.

is less marked; BAL gave complete protection when used 10 minutes after application of 0.4 mg. of lewisite.

Effect of time between contamination and decontamination. As the length of time elapsing between application of the vesicant and treatment with BAL was increased, the degree of decontamination was diminished. In hot weather (above 85°F.), 0.4 M. BAL was virtually ineffective in preventing vesication by 95 μg. of lewisite applied 90 minutes previously.

Effect of environmental factors. While we did not make a controlled study of the effects of temperature and relative humidity in decontamination by lewisite, we collected considerable data which demonstrate that these factors are important. Table II lists five respresentative experiments, carried out under identical conditions except for those of climate. The untreated applications of lewisite produced 95–100% blisters on each day. It would seem that the indoor temperature and relative humidity were more important than the outdoor temperature (cf. tests on 1/20/43 and 8/17/43).

TABLE II

Effect of climatic conditions on the decontamination of Lewisite by BAL

Dose of Lewisite: 95 μg. Interval Lewisite to BAL: 40 minutes. Concentration of BAL: 0.4 M.

DATE	INDOOR TEMP.	INDOOR R.H.	MEAN OUTDOOR TEMP.	DECON-TAMINANT	NO OF MEN	% ERYTHEMAS	% BLISTERS
	°F.	%	°F.				
1/20/43	63	37	20	BAL	19	10	5
7/20/43	86	50	80	BAL	119	55	40
8/17/43	69	59	64	BAL	101	13	2
9/14/43	65	80	50	BAL	72	12	4
10/12/43	65	80	68	BAL	50	18	2

These differences probably reflect the variations with temperature in rate of penetration and subsequent rate of enzyme inhibition by lewisite.

Effect of concentration of BAL. It was found that concentrated solutions of the dithiols were more effective than the more dilute preparations in the treatment of lewisite on human skin, probably because of the increased amount of dithiol per unit area of skin in the case of higher concentrations. This finding was demonstrated more strikingly with some of the BAL derivatives than with BAL itself (Table III).

Water-soluble BAL derivatives: Since the solubility of BAL in water is low, its uses in aqueous solution are limited. Thus a number of derivatives of BAL which are appreciably soluble in water are of interest, since they possess therapeutic properties similar to that of BAL, are less toxic, and have a less objectionable odor.

Four of these water-soluble compounds, KD-2, KD-8, KD-9, and NDR 475, were tested more extensively than the others, and will be given special consideration in this report.

KD-2. This compound, the diethanolaminomethyl thioether of BAL, was the first and perhaps the most effective of the water-soluble derivatives submitted. As will be seen in Table III, its decontaminating efficacy is nearly equal to that of BAL. Furghermore, KD-2 is less toxic; its LD-50 for mice (intraperitoneal) is 350 mg./kg., over three times as great as that of BAL.

The effect of concentration of the decontaminant in the vehicle is demonstrated in the second part of Table III. The 0.4 M. solution of KD-2 in water was much less effective than the 0.8 M. solution in the decontamination of 0.2 mg. of lewisite after 2 and 5 minutes.

The decontaminating value of KD-2 was nearly negligible after storage for six weeks at 60°C., in contrast to the behavior of BAL, which retains its effectiveness under such conditions.

TABLE III

Decontamination of Lewisite by KD-2

DECONTAMINANT	INTERVAL	NO. OF MEN	ERYTHEMAS	BLISTERS
Dose of Lewisite: *ca.* 50 μg. Indoor temp.: 66°F Indoor R H.: 48%				
	min			
KD-2, 0.8 M. in propylene glycol	20	18	0	0
BAL, 0.8 M. in propylene glycol			0	0
KD-2, 0.8 M in propylene glycol	40	19	1	1
BAL, 0.8 M in propylene glycol			0	0
KD-2, 0.8 M in propylene glycol	60	19	7	1
BAL, 0.8 M in propylene glycol			0	0
Dose of Lewisite: 0.2 mg. Indoor temp: 59°F. Indoor R H.: 72%				
KD-2, 0.4 M. in water	2	20	14	6
KD-2, 0.8 M in water	2	20	4	0
KD-2, 0.4 M. in water	5	20	20	16
KD-2, 0.8 M. in water	5	20	5	0

KD-8. This compound, prepared from sarcosine, formaldehyde, and BAL, was the most extensively tested of all of the water-soluble derivatives. Although it was a good decontaminant of lewisite on human skin and was even less toxic than KD-2 (LD-50 for mice intraperitoneal injection > 400 mg./kg.), it was not as effective as BAL.

A few tests with KD-8 were carried out in which the compound was precipitated as the crystalline sodium salt and then redissolved in water before testing. Table IV gives the results of an experiment comparing the reprecipitated compound with KD-8 in freshly prepared solution. It will be seen that the former preparation was less effective.

KD-9. The glycine analogue of KD-8 was definitely less effective than BAL. However, precipitation of the compound resulted in no loss in decontaminative

action of KD-9, as the folowing example shows (Table V). The solid KD-9 was determined to be about 90% pure on the basis of sulfur content.

NDR 475. The tests with this preparation were limited to three comparisons with BAL and two with KD-8. NDR 475 appears to be about equal to KD-8

TABLE IV

Tests of reprecipitated KD-8

Dose of Lewisite: *ca.* 90µg. Indoor temp.: 55°F. Indoor R.H.: 54%.

DECONTAMINANT	INTERVAL, LEWISITE TO KD-8	NO OF MEN	ERYTHEMAS	BLISTERS
	min			
KD-8, 0 8 M. in water, freshly prepared	40	20	1	0
Untreated control			20	20
KD-8, 0.8 M. in water, reprecipitated	40	21	10	9
Untreated control			20	20
KD-8, 0 8 M. in water, freshly prepared	60	18	4	2
Untreated control			18	15
KD-8, 0 8 M. in water, reprecipitated	60	20	9	7
Unteated control			20	19

TABLE V

Decontamination of Lewisite by KD-9

Dose of Lewisite: 95 µg. Indoor temp.: 67°F. Indoor R.H.: 30%.

DECONTAMINANT	INTERVAL, LEWISITE TO KD 9	NO OF MEN	ERYTHEMAS	BLISTERS
	min			
KD-9, 0 8 M. in water, freshly prepared	60	36	16	7
Untreated control			36	36
KD-9, 0 8 M. in water, reprecipitated	60	38	15	8
Untreated control			38	37
BAL, 0.4 M. in ointment	60	25	1	1
Untreated control			25	23

and inferior to BAL in the decontamination of lewisite. The results of three of the tests are listed in Table VI.

Other water-soluble BAL derivatives. The rest of the water-soluble compounds were not tested as completely as were the four previously mentioned. The results of the experiments with these other preparations are listed in Table VII. All of

them were effective except for KD-10 (sodium salt of N-phenyl-N-sulfomethylaminomethyl thioether of BAL), and NDR 406 and 407, in which both thiol groups of BAL were condensed with formaldehyde-acetamide compounds.

The entries in Table VII are arranged in chronological order; those between lines were tested on the same day.

Tests of related dithiols: The results of our experiments with 26 other dithiols are presented in Table VIII. It will be noticed that several compounds were not compared with BAL, either on the same men or on different men tested on the same day. Most of these experiments were of a preliminary nature, and the compounds examined in such tests were not recommended to us for more thorough testing because of toxicity, instability, or difficulty of synthesis. Dimercaptopropyl chloride (NDR 320), dimercaptopropylamine (NDR 347), and dimercaptobenzene (NDR 378) were three such compounds.

TABLE VI

Decontamination of Lewisite by NDR 475

Dose of Lewisite: 95 μg.

DECONTAMINANT	INTERVAL	NO OF MEN	ERYTHEMAS	BLISTERS	INDOOR TEMP. & R H.
	min				
NDR 475, 0 4 M. in propylene glycol	40	19	1	0	69°
BAL, 0.4 M. in propylene glycol			1	0	59%
NDR 475, 0.4 M. in water	40	9	1	0	69°
KD-8, 0 4 M. in water			2	0	59%
NDR 475, 0.4 M. in propylene glycol.	60	14	13	9	81°
BAL, 0.4 M. in propylene glycol			9	4	75%

As in Table VII, the entries in Table VIII are arranged in chronological order, with lines separating tests made on different days.

Most of these compounds were prepared by Dr. W. A. Lazier and coworkers at the DuPont Experimental Station.

Tests with other sulfur compounds: Monothiols. Thioglycollic acid, thioacetamide, *o*-aminothiophenol, N-2-mercaptoethylphthalimide, mercaptobenzothiazole, and 2-mercapto-4-hydroxy-thiazole were ineffective in decontamination of 0.1 mg. of lewisite. Monothioethylene glycol was moderately effective, although far inferior to BAL.

Thioureas and Dithiocarbamates. Dithiobiuret and ethyl dithioallophanate both of which could be classed as dithiols, were moderately good decontaminants of lewisite. Allyl thiourea and isobutyl thiourea were relatively poor, while thiourea and n-butyl thiourea were completely ineffective. Dimethylaminomethyl dimethyldithiocarbamate was the most effective of five dithiocarbamates examined; it was much less effective than BAL. Sodium diethyldithiocarba-

TABLE VII

Decontamination of Lewisite by other water-soluble BAL derivatives

Dose of Lewisite: *ca.* 90 μg. Interval, lewisite to treatment: 40 minutes.

DECONTAMINANT	NO. OF MEN	ERYTHEMAS	BLISTERS	INDOOR TEMP. AND R.H.
KD-7, 1.6 M. in water	23	0	0	73°F.
Untreated control		23	22	57%
KD-10, 1.6 M. in water	26	19	18	73°
Untreated control		26	26	57%
KD-11 (NDR 288), 1.6 M. in water	20	0	0	73°
Untreated control		20	20	57%
KD-15, 1.6 M. in water	20	11	1	73°
Untreated control		20	20	57%
KD-37, 0.8 M. in water	20	0	0	49°
Untreated control		20	20	51%
KD-38, 0.8 M. in water	20	2	1	49°
Untreated control		20	20	51%
KD-39, p.8 M. in water	20	0	0	49°
Untreated control		20	19	51%
Methylglucitylammonium salt of BAL (NDR 290), 0.8 M. in water	17	0	0	49°
Untreated control		17	17	51%
Ethylenediamine salt of BAL KD-43, 0.8 M. in water	21	1	0	49°
Untreated control		21	21	51%
Diethanolammonium salt of BAL KD-44 (NDR 289), 0.8 M. in water	19	0	0	49°
Untreated control		19	19	51%
KD-11 (NDR 288), 0.8 M. in water	20	3	2	49°
Untreated control		20	20	51%
BAL, 0.4 M. in ointment	20	0	0	49°
Untreated control		20	19	51%
Dose of Lewisite: 95 μg.				
Bis-S(acetamidomethyl) thioether of BAL (NDR 406), 0.4 M. in propylene glycol	16	15	15	87°
BAL, 0.4 M. in propylene glycol		8	6	50%
Bis-S(ethylacetamidomethyl) thioether of BAL (NDR 407), 0.4 M. in propylene glycol	13	13	13	87°
BAL, 0.4 M. in propylene glycol		7	4	50%

TABLE VIII

Decontamination of Lewisite by other dithiols

Dose of Lewisite: *ca.* 90 μg.

DECONTAMINANT	INTERVAL	NO. OF MEN	ERYTHEMAS	BLISTERS	INDOOR TEMP. & R.H.
	min.				
N-(2,3-Dimercaptopropyl) aniline hydrochloride (NDR 225), 0.2 M. in 87% alcohol)	60	10	0	0	72°
Untreated control			10	10	42%
BAL, 0.4 M. in propylene glycol	60	10	2	2	72°
Untreated control			10	8	42%
Formaldehyde mercaptol of BAL (KD-17), 0.4 M. in propylene glycol	60	20	9	3	59°
Untreated control			20	19	78%
Dimethylaminomethyl thioether of BAL KD-12, 0.4 M. in ointment	60	20	18	17	59°
BAL, 0.4 M. in ointment			7	3	78%
2,3-Dimercaptopropyl ether (NDR 293), 0.4 M. in ointment	40	20	18	7	49°
Untreated control			20	20	51%
1,3-Dimercapto-2-propanol (NDR 131), 0.4 M. in ointment	40	17	11	2	49°
Untreated control			17	16	51%
1,6-Hexane dithiol (NDR 139), 0.4 M. in ointment	40	20	17	13	49°
Untreated control			20	20	51%
1,3-Propane dithiol (NDR 132), 0.4 M. in ointment	40	20	14	7	49°
Untreated control			20	18	51%
Diethanolaminomethyl thioether of 1,3-propane dithiol (water-soluble derivative of NDR 132—same as NDR 411), 0.8 M. in water	40	19	0	0	49°
Untreated control			19	19	51%
BAL, 0.4 M. in ointment	40	42	3	0	49°
Untreated control			41	39	51%
Dithiooxamide (NDR 313), 0.25 M. in formamide	40	20	3	3	69°
Untreated control			17	17	24%

TABLE VIII—*Continued*

DECONTAMINANT	INTERVAL	NO. OF MEN	ERYTHEMAS	BLISTERS	INDOOR TEMP. & R.H.
	min.				
2,3-Dimercaptopropionic acid (NDR 317), 0.8 M. in propylene glycol........	40	20	0	0	69°
Untreated control....................			20	18	24%
2,3-Dimercaptopropionic acid (NDR 317), sodium salt, 0.8 M. in water (pH 6)................................	40	20	2	0	69°
Untreated control....................			20	20	24%
Methyl 2,3-dimercaptopropionate (NDR 318), 0.4 M. in ointment........	40	20	9	3	69°
Untreated control....................			20	20	24%
2,3-Dimercaptopropylamine (NDR 347), 0.8 M. in water......................	40	11	2	0	72°
Untreated control....................			11	11	31%
1,2-Dimercaptobenzene (NDR 378), sodium salt, 0.7 M. in water (pH 7)....	40	10	8	2	76°
Untreated control....................			10	10	28%
2,3-Dimercaptopropyl chloride (NDR 320), 0.8 M. in "Cellosolve"..........	40	10	5	1	63°
Untreated control....................			10	10	55%
Phenylethanedithiol (NDR 476), 0.4 M. in dioxane..........................	40	29	27	23	87°
BAL, 0.4 M. in dioxane................			7	5	50%
3,4-Dimercaptotetrahydrothiophene-1-dioxide (NDR 346), 0.4 M. in dioxane..	40	10	10	9	87°
BAL, 0.4 M. in dioxane................			9	7	50%
2,3-Dimercaptopropylurea (NDR 399), 0.4 M. in propylene glycol...........	40	14	13	12	87°
BAL, 0.4 M. in propylene glycol.......			8	4	50%
2,3-Dimercaptopropylurethane (NDR 400), 0.4 M. in propylene glycol........	40	14	14	14	87°
BAL, 0.4 M. in propylene glycol.......			9	8	50%
2,3-Dimercaptopropyl methyl ether (NDR 391), 0.4 M. in propylene glycol.	40	21	7	1	69°
BAL, 0.4 M. in propylene glycol.......			1	0	59%
p-Dimethylaminobenzaldehyde mercaptol of BAL (KD-81), 0.4 M. in ointment................................	40	20	6	0	69° 59%

TABLE VIII—*Continued*

DECONTAMINANT	INTERVAL	NO OF MEN	ERYTHEMAS	BLISTERS	INDOOR TEMP & R H
	min				
2,3-Dimercaptopropyl acetate (NDR 230), 0.4 M. in propylene glycol	40	23	11	5	65°
BAL, 0 4 M in propylene glycol			1	0	80%
2,3-Dimercaptopropyl propionate (NDR 607), 0 4 M in propylene glycol	40	11	0	0	65°
BAL, 0 4 M. in propylene glycol			0	0	80%
2,3-Dimercaptopropyl butyrate (NDR 608), 0 4 M in propylene glycol	40	10	0	0	65°
BAL, 0 4 M in propylene glycol			0	0	80%
2,3-Dimercaptopropyl isopropyl ether (NDR 609), 0 4 M. in propylene glycol	40	13	1	0	65°
BAL, 0 4 M. in propylene glycol			2	0	80%
2,5-Dimercaptothiadiazole (NDR 610), 0 4 M. in propylene glycol	40	16	14	2	65°
BAL, 0 4 M. in propylene glycol			7	1	80%
3,4-Dimercapto-1-butanol (NDR 644), 0 4 M. in propylene glycol	40	42	5	0	73°
BAL, 0 4 M in propylene glycol			1	0	46%

mate, sodium diethanoldithiocarbamate, sodium pentamethylenedithiocarbamate, and bis-(dimethylthiocarbamyl)sulfide were relatively ineffective.

Thioacids. Ten thioacids, salts, and esters, were found to be of no particular value in lewisite therapy. Some protection was offered by potassium tetrahydrofurfurylxanthate, potassium thiolacetate, dithioacetic acid, potassium thiolaurate, potassium *m*-nitrothiolbenzoate, sodium dithio-β-resorcylate, sodium *p*-toluene thiosulfonate, potassium dithiooxalate, ethylenediamine thiosulfate, and vinyl thiolacetate-diethyl fumarate copolymer; however, our results do not suggest that any of these would seriously challenge the dithiols as decontaminants of lewisite.

DISCUSSION. With the exception of some of the water-soluble derivatives, only six other dithiols seemed to be as effective as BAL: 2,3-dimercaptopropylaniline hydrochloride (NDR 225), 2,3-dimercaptopropylamine (NDR 347), 2,3-dimercaptopropionic acid (NDR 317), 2,3-dimercaptopropyl propionate (NDR 607), 2,3-dimercaptopropyl butyrate (NDR 608), and 2,3-dimercaptopropyl isopropyl ether (NDR 609).

Among the water-soluble derivatives, KD-2, KD-7, KD-11, KD-37, KD-38 KD-39, NDR 290, KD-43, and KD-44, appeared to decontaminate 0.1 mg. amounts of lewisite practically as effectively as BAL. Only KD-2 was tested

extensively, however; had the others been examined as thoroughly as KD-2, KD-8, and KD-9, particularly in regard to their efficacies in hot weather, many of them might have been found to be inferior to BAL. Furthermore, it should be noted that nearly all of these derivatives were tested as 0.8 M. solutions rather than 0.4 M., in order to provide an equivalent number of free SH groups.

KD-8 and KD-9, although not quite as effective as BAL, are of interest because they may be readily prepared as crystalline solids, whereas some of the other water-soluble compounds of similar structure formed oils or gummy amorphous masses upon precipitation (4).

In view of the findings of this and other laboratories on the efficacy of BAL as a decontaminant of human skin after application of lewisite, and the findings of other investigators on BAL as a therapeutic agent systemically and in the eye (1), it seems to be the most satisfactory of the dithiols, its high toxicity notwithstanding.

SUMMARY

1. The vesicant action of 0.1 mg. of lewisite on human skin can be prevented more effectively by treatment with 2,3-dimercaptopropanol (BAL) than by most of the other dithiols tested.

2. The effectiveness of BAL and other dithiols is dependent largely upon (1) the dose of lewisite used, (2) the interval between contamination and decontamination, (3) the concentration of the dithiol in the vehicle, and, especially, (4) the temperature and relative humidity of the immediate envioronment of the test subject.

3. A number of water-soluble derivatives of BAL have been tested, several of which are nearly as effective as BAL.

4. Monothiols, thiourea derivatives, and thioacids were of little effect in the decontamination of lewisite on human skin.

REFERENCES

1. Waters, L. L., and Stock, C. C.: Science, 102: 601, 1945.
2. Peters, R. A., Stocken, L. A., and Thompson, R. H. S.: Nature, 156: 617, 1945
3. Bloom, W., Murrary, R. G., Savit, J., and Thomson, J. F.: Unpublished data.
4. Kharasch, M. S., and Weinhouse, S.: Unpublished data.

THE EFFECT OF SODIUM CAPRYLATE ON THE CARDIAC OUTPUT OF THE ISOLATED FROG HEART

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Received for publication September 11, 1946

The findings of Hall and Waldman (1) that sodium caprylate, when administered intravenously to cats, produces an electrocardiographic picture similar to that produced by toxic doses of digitalis prompted a series of experiments to investigate the possibility that sodium caprylate might have a digitalis-like action on the contraction of the heart. This report is on a study of the effect of sodium caprylate on the cardiac output of isolated frog hearts.

METHODS. A glass cannula 6.5 cm. long with a U-shaped curve on its free end was inserted through the left aortic arch into the bulbus arteriosus, and a glass cannula connected by rubber tubing to a 2 ml. reservoir was tied into the inferior vena cava. All other vessels were ligated, the heart was removed from the frog and supported by clamps attached to the cannulae. The height of the reservoir was adjusted so that its top was 4.5 cm. above the heart. It was kept filled to overflowing from a large reservoir so that the venous pressure was always 4.5 cm. of water. The heart rate was counted for 30 seconds at regular intervals and the output simultaneously collected from the curved aortic cannula. Stroke volumes were calculated. The perfusion fluid was glucose-Ringers or solutions of sodium caprylate made up in glucose-Ringers.

Graphic records of some experiments were made using a small cardiometer. The isolated heart was placed in the cardiometer in such a manner that the cannulae projected from the top of the vessel. The cardiometer was then sealed with plasticene and connected to a sensitive tambour 1 cm. in diameter.

RESULTS. When the hearts were perfused with glucose-Ringers, they dilated and failed spontaneously after one to three hours. A cardiac output of 50 per cent or less of the maximum attained early in the experiment was used as a criterion of failure. When sodium caprylate was added to the perfusion fluid in concentrations ranging from 0.000625 to 0.025 molar it caused a marked increase in the cardiac output of the failing hearts. This increase occurred within one to two minutes but was usually of rather short duration and the hearts would again fail after a period of 15 to 20 minutes of caprylate perfusion. The hearts showed varying sensitivity to caprylate, a concentration highly effective on one having little or no effect on another. The effectiveness also varied with the degree of failure of the hearts as shown by comparing results in table 1 with those in table 2. There was comparatively little effect on fresh hearts. Because of this variability the data have been arranged to show the per cent increase in cardiac output above the level to which the output had declined just before the addition of caprylate to the perfusion fluid. Tables 1 and 2 indicate the degree of failure attained by each heart at the time the caprylate was added and the resulting percentage increase in the cardiac output. The course of a typical experiment

TABLE 1

The effect of sodium caprylate on the cardiac output of failing isolated frog hearts

FROG NUMBER	GLUCOSE-RINGERS		SODIUM CAPRYLATE		
	Maximum C.O.*	Pre-caprylate C.O.*	Molar concentration	Maximum C.O.*	% increase
47	10.7	3.0	0.000625	5.6	87
48	12.4	4.2	0.000625	4.0	−4
45	15.8	1.5	0.00125	5.6	273
46	19.3	3.6	0.00125	9.2	156
11	16.2	0.4	0.0025	8.4	2000
34	14.3	4.4	0.0025	8.2	86
42	15.4	6.0	0.0025	9.6	60
42	15.4	1.0	0.0025	9.6	860
44	6.2	2.7	0.0025	3.3	22
46	19.3	2.0	0.0025	8.2	310
4	12.4	6.0	0.00312	11.0	83
6	16.4	3.9	0.005	7.2	85
9	11.0	5.2	0.005	11.2	115
9	11.0	1.6	0.005	3.0	87
10	10.0	3.6	0.005	13.6	280
11	16.2	6.2	0.005	17.0	174
12	10.3	0.4	0.005	1.8	350
14	11.1	0.4	0.005	1.5	275
18	12.0	1.6	0.005	7.6	375
19	26.0	0.7	0.005	13.6	1843
24	18.4	4.0	0.005	9.6	140
4	12.4	5.1	0.00625	11.8	131
10	10.0	0.1	0.0075	11.4	11300
4	12.4	4.0	0.0125	13.2	230
3	14.6	5.0	0.025	18.0	260

* All cardiac outputs are in mls. per minute.

TABLE 2

The effect of sodium caprylate on the cardiac output of fresh isolated frog hearts

FROG NUMBER	GLUCOSE-RINGERS		SODIUM CAPRYLATE		
	Maximum C.O.*	Precaprylate C.O.	Molar concentration	Maximum C.O.	% increase
5	5.4	5.4	0.0025	6.4	18
10	10.0	9.2	0.0025	10.6	15
12	10.3	8.7	0.0025	9.0	3
13	7.8	4.5	0.0025	8.0	78
7	17.1	17.0	0.005	17.4	2
8	13.0	8.2	0.005	12.5	52
15	9.0	9.0	0.005	11.4	26
16	10.4	10.4	0.005	11.6	11
22	9.2	9.2	0.005	9.8	6
28	6.4	6.4	0.005	8.8	38
2	13.4	13.4	0.025	18.2	36
2	13.4	8.8	0.025	15.2	73
4	12.4	12.4	0.025	14.1	14

* All cardiac outputs are in mls. per minute.

is shown in figure 1 in which the cardiac output, stroke volume and heart rate are plotted against time.

The venous pressure and peripheral resistance were kept constant and it was noted that the caprylate had no marked or consistent effect on the heart rate except in large concentrations which slowed the heart as shown in figure 1. Therefore the increase in cardiac output must have been due to an increase in

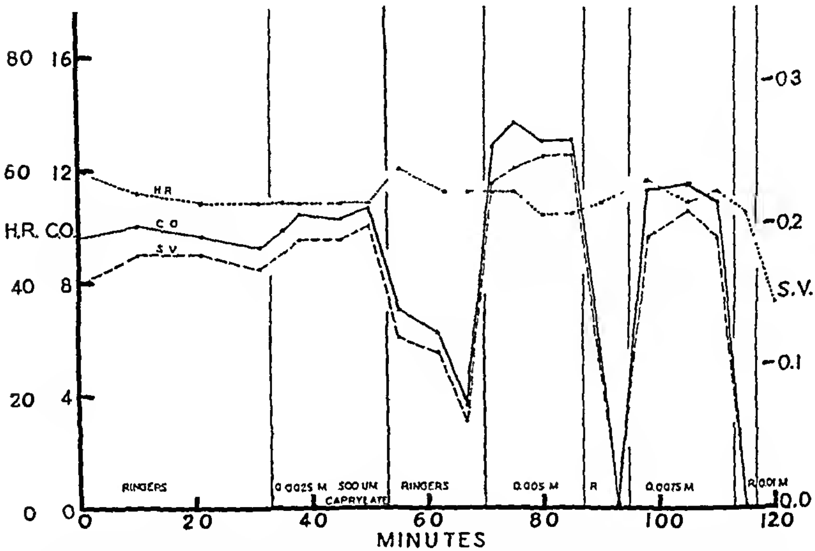

FIG. 1. CHANGES IN HEART RATE, CARDIAC OUTPUT AND STROKE VOLUME WITH TIME WHEN AN ISOLATED FROG HEART IS PERFUSED WITH GLUCOSE-RINGERS SOLUTION OR VARIOUS CONCENTRATIONS OF SODIUM CAPRYLATE MADE UP IN GLUCOSE-RINGERS

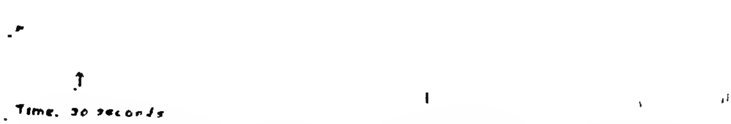

FIG. 2. CARDIOMETER RECORD OF AN ISOLATED FROG HEART PERFUSED WITH GLUCOSE-RINGERS WITH 0.005 MOLAR SODIUM CAPRYLATE ADDED AT THE ARROW

The upstroke indicates diastole, the downstroke systole

stroke volume. It was found that in all cases the changes in stroke volume closely followed the changes in cardiac output when they were plotted as in figure 1. This change in stroke volume was confirmed by means of the graphic records obtained by use of the cardiometer. Figure 2 illustrates the increase in systolic emptying of the heart after addition of sodium caprylate to the perfusion fluid.

DISCUSSION. Clark (2) found that various higher fatty acids improved the

functioning of the hypodynamic frog heart. Lissak (3) also found that sodium oleate had a stimulating effect on the fatigued frog heart. Clark, Gaddie and Stewart (4) found that frog hearts poisoned with iodoacetate would contine to function if supplied with any fatty acid from proprionic to decoic. Furchgott and Shorr (5) using a mammalian smooth muscle preparation showed that fatty acids with an even number of carbon atoms were more effective in bringing about recovery of contractility after its failure due to substrate removal than were fatty acids with an odd number of carbon atoms. Such evidence indicates that the fatty acids may be utilized as sources of energy by frog heart and mammalian smooth muscle. Our work suggests an action of caprylate on frog muscle apart from its usefulness as a source of energy inasmuch as glucose was always present in the perfusion fluid. The brief period of action remains unexplained but may be considered additional evidence that caprylate is not acting as a substrate in this instance.

The action of caprylate in strengthening the contractions of the heart with consequent improvement in systolic ejection and cardiac output does suggest the action of digitalis. A further resemblance is its far more effective action on the weak and failing heart than on the fresh heart. Since the venous pressure was constant the effect must have been on the heart muscle itself. This is reflected in the increased stroke volume and the more complete systolic emptying as shown by the graphic records and by visual examination of the hearts during the experiment. It was noted that the heart beat more forcefully and emptied more completely and in many cases the degree of dilation was reduced during the period of caprylate perfusion, only to have this improvement cease within two minutes after changing back to glucose-Ringers.

SUMMARY

Sodium caprylate added to glucose-Ringers in concentrations between 0.000625 and 0.025 molar has a stimulating effect on the cardiac output of the isolated frog heart under conditions of constant venous pressure and peripheral resistance. The effect is much more marked in hearts that have failed than on fresh hearts. The effect is brought about by an increase in stroke volume apparently by improving the contractility of the muscle.

BIBLIOGRAPHY

1. Hall, V. E., and Waldman, M. S.: This Journal, in press.
2. Clark, A. J.: Journ. Physiol., 47: 66, 1914.
3. Lissák, K.: Arch. f. Exp. Path. u. Pharmakol., 181: 233, 1936.
4. Clark, A. J., Gaddie, R., and Stewart, C. P.: Journ. Physiol., 90: 335, 1937.
5. Furchgott, R. F., and Shorr, E.: Proc. Soc. Exp. Biol. Med., 61: 280, 1946.

THE CHRONIC ORAL TOXICITY OF DDT (2,2-BIS (p-CHLOROPHENYL-1,1,1-TRICHLOROETHANE)[1]

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Received for publication September 14, 1946

Recent studies on the pharmacology of DDT have treated with short-term toxicity experiments on mammals (1–6), with its storage in animal tissues (7, 8, 9), and with its excretion (8, 10, 11). No study of the lifetime effects of DDT on laboratory animals has been reported. Since long-term feeding experiments with other substances in this laboratory have revealed deleterious effects which would not have been seen in experiments conducted for shorter periods of time, it seemed advisable to feed DDT for the lifetime of the rat.

PART I. TWO-YEAR EXPERIMENTS. *Method.* Two experiments were conducted in which groups of weanling rats (21 days) from our colony of Osborne-Mendel strain were started on diets containing a commercial preparation of DDT composed of 81.8% p,p isomer and 18.2% o,p isomer. In the first experiment, started early in 1943 when our supply of DDT was small, 5 groups of 12 male rats were fed on diets containing respectively 0, 100, 200, 400 and 800 p.p.m. DDT incorporated in a 10% corn oil solution. In a second experiment, started about a year later, 7 groups of 24 rats, equally divided between the sexes, were fed on diets containing respectively 0, 200, 400, 600 and 800 p.p.m. DDT incorporated in a 10% corn oil solution, and 600 and 800 p.p.m. dry DDT for comparison with the oil solutions. Ground commercial rat biscuits with 1% added cod liver oil served as the basic diet. Litter mates were selected and assigned to the various groups in both experiments according to a randomized design of experiment (balanced incomplete blocks (12)). All animals were kept in individual cages in a room with controlled temperature and humidity and were given free access to their respective diets and water. Body weights and food consumption were determined at weekly intervals.

RESULTS. Since the second experiment involved a much larger number of animals than the first, the following discussion of results will be confined to the former except that mention will be made to any differences which occurred in the two experiments.

The production of tremors. The first noticeable effect of DDT was a hyper-irritability as shown by a sensitivity of the rats to stimuli and the appearance of fine tremors, especially in those animals on 600 and 800 p.p.m. DDT in the diet. These tremors developed earlier in the female rats than they did in the male rats. The rats with severe tremors became progressively worse to the point of convulsions and death. The rats on the dosage levels of 600 and 800 p.p.m. DDT that survived for the first year exhibited only moderate tremors notably during the early part of the exposure and later appeared to recover. In the group

[1] A portion of the funds used in this investigation was supplied by a transfer, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Division of Pharmacology of the Food and Drug Administration.

on 400 p.p.m. DDT, 10 out of 24 animals showed extreme tremors before death; 8 of these 10 were female rats. Severe tremors were noted in 2 female rats in the group on 200 p.p.m. of DDT.

Since a large amount of DDT may be stored in the body tissues (7), a study was made to show the effect of starvation on the metabolism of the stored DDT. At each dosage level the DDT-containing diet was withdrawn from 3 rats which had been on the experiment for 18 months and had exhibited no severe nervous symptoms up to that time. Within 24 hours after withdrawal of all food the rats formerly on 600 and 800 p.p.m. DDT showed marked tremors. Those formerly on 200 and 400 p.p.m. DDT showed increased irritability. In a similar study to show the effect of partial starvation the DDT-containing diet was exchanged for the control diet reduced to a fourth of the daily food requirement. The partial starvation did not produce characteristic DDT tremors in rats from any group.

The effect on food consumption and growth. It was shown in a paired feeding experiment (7) that 800 p.p.m. DDT produces a significant retardation in growth without a corresponding decrease in food intake. Likewise in this experiment there was no significant ($p = .05$, or less, is significant) difference between the food intake of any group of animals and that of the controls.

Since many animals on the 800 p.p.m. DDT died early, the interval of the first 12 weeks on the experimental diet was selected as the first period of study for a comparison of growth data from all groups. During this period the retarding effect of DDT on growth was significant for the female rats at concentrations of 400 p.p.m., or more, and for the male rats only at the 800 p.p.m. DDT in the diet. There was no increase in the effect on growth of the female rats as the concentration of DDT increased from 400 to 800 p.p.m.

In order to study the effect of DDT on growth for a longer period, a second interval of the first year on the experimental diet was selected. At the end of the year only 1 female rat was living from the two groups on 800 p.p.m. DDT. An analysis (table 1) shows that there was a retardation of growth in all groups of female rats; however, the value for the group on 200 p.p.m. remained nonsignificant. Slight retardation of growth occurred in the male rats on 600 and 800 p.p.m. DDT. The difference between the group on 800 p.p.m. dry DDT and the controls was significant at 3 months. Because of the small number of rats surviving for a year, this difference became not significant.

As shown in table 1 the dissolving of DDT in corn oil did not change the toxic effect of DDT on growth at the concentrations used in this experiment.

The effect on mortality. The mortality rate of the rats fed diets containing DDT was related to the dosage of DDT and to the sex of the rats (table 2). DDT at 800 p.p.m. in the diet produced severe nervous symptoms which were followed by death in many animals within the first few months. Severe nervous involvement occurred earlier in the female rats than in the male rats and death occurred earlier, therefore, in them. At the 800 p.p.m. level only 1 female rat lived for as long as a year. There was an increase in mortality rate for the female rats over that of the controls on all concentrations of DDT. The per cent difference in

TABLE 1

Mean gain in weight of rats fed diets containing DDT (second experiment)

TIME	DOSAGE OF DDT	SEX	NO. OF ANIMALS	MEAN GAIN IN WEIGHT
months	*p.p.m.*			
3	0	M	11	310.2 ±13.3
		F	12	205.3 ± 6.8
	200	M	12	300.8 ± 9.5
		F	12	203.7 ± 6.8
	400	M	12	316.6 ± 5.3
		F	12	177.3 ± 6.4†
	600	M	12	279.9 ±11.4
		F	12	178.8 ±60.0†
	600 Dry	M	12	280.0 ± 9.9
		F	10	176.7 ± 4.1†
	800	M	12	273.7 ±14.8
		F	12	172.8 ± 7.7†
	800 Dry	M	10	255.7 ± 8.5†
		F	9	177.2 ± 7.6†
12	0	M	10	486.6 ±18.9
		F	11	293.5 ±10.7
	200	M	9	488.1 ±26.5
		F	10	282.0 ±10.9
	400	M	10	537.8 ±24.2
		F	9	253.7 ±10.8*
	600	M	11	481.4 ±23.2
		F	5	240.4 ±14.9*
	600 Dry	M	11	463.6 ± 8.7
		F	3	238.7 ± 4.4†
	800	M	10	473.7 ±21.0
		F	1	
	800 Dry	M	6	459.8 ±12.9
		F	0	

* $p < .05 - > .01$.

† $p < .01$.

the mortality rate between the experimental groups of females and those of the control at a year on the experimental diets ranged from 8.3% for those on 200 p.p.m. DDT to 91.7% for those on 800 p.p.m. At the end of the experimental

period the differences appeared to have a definite break at 400 p.p.m.; however, because of the small number of rats the difference at 600 p.p.m. was not significant. When all groups of female rats on 400 to 800 p.p.m. DDT were compared jointly with those on the 200 p.p.m. DDT and the control group the difference in mortality rate was found to be highly significant.

Fewer male rats were living at the end of the experimental period in the groups

TABLE 2

Per cent mortality of rats fed diets containing DDT

DOSAGE OF DDT	SEX	12 MOS.	18 MOS.	24 MOS.
		First experiment		
p.p.m.				
0	M	25	58.3	66.7
100	M	33.3	50	58.3
200	M	33.3	41.7	66.7
400	M	25	58.3	83.4
800	M	75	83.4	91.7
		Second Experiment		
0	M	16.6	50	75
	F	8.3	25	58.3
200	M	25	33.3	50
	F	16.6	25	83.4
400	M	16.6	75	91.7
	F	25	50	100
600	M	8.3	33.3	83.4
	F	41.7	66.7	83.4
600 Dry	M	8.3	41.7	83.4
	F	58.3	66.7	91.7
800	M	16.6	41.7	83.4
	F	91.7	91.7	100
800 Dry	M	50	66.7	83.4
	F	100	100	100

on 400 p.p.m. or more of DDT than in the control group, but the difference between groups was not significant. There was no definite relation of the mortality rate to the dosage of DDT as mentioned above for the female rats. In the first experiment the death of 5 male rats on 800 p.p.m. DDT within the first two weeks accounts for the difference in mortality in the 2 experiments. No explanation can be advanced for the death of these rats early in the experimental

period. The paired feeding experiment referred to previously (7) gave results similar to the second experiment.

There was no difference between the mortality rate of the rats on diets with the DDT dissolved in corn oil, and that of rats on diets with the same concentration of dry DDT.

The effect on the weights of the liver, kidneys and spleen. At autopsy it was noted that the livers, and to a lesser extent the kidneys and the spleens, of the experimental animals were larger than those of the controls. Weighing these

TABLE 3

The effect of chronic ingestion of DDT on the weight of the livers, kidneys and spleens of rats

DOSAGE OF DDT	SEX	NO. OF RATS	MEAN WEIGHT (GRAMS PER KGM. OF BODY WEIGHT)		
			Liver	Kidneys	Spleen
p.p.m.					
0	M	6	25.6 ±2.9	6.6 ±0.5	1.1 ±0.2
	F	7	32.7 ±3.5	7.4 ±0.4	1.7 ±0.3
100	M	4	32.2 ±1.6	7.4 ±0.5	1.6 ±0.1
200	M	7	33.2 ±2.6	6.3 ±0.3	1.9 ±0.2
	F	9	48.7 ±3.8†	8.5 ±0.4	2.1 ±0.4
400	M	6	39.9 ±2.9†	6.8 ±0.2	1.4 ±0.4
	F	7	42.7 ±2.3*	8.3 ±0.8	1.7 ±0.4
600	M	7	41.4 ±3.5†	8.5 ±0.5*	2.0 ±0.4
	F	4	67.3 ±3.3†	9.1 ±0.5*	2.3 ±0.7
600 Dry	M	5	44.1 ±6.1*	8.5 ±0.3*	1.3 ±0.6
	F	4	60.6 ±2.1†	9.2 ±0.7*	1.8 ±0.4
800	M	8	47.3 ±3.7†	8.3 ±0.4*	1.6 ±0.2
800 Dry	M	4	44.2 ±1.5†	8.7 ±0.5*	2.0 ±0.3

* p. <.05 – >.01.
† p. <.01.

organs of rats, which had been on diets containing DDT for 18 months, or more, confirmed this observation, the results of which are shown in table 3. The differences in weights of these organs increase with the increase in concentration of DDT in the diets. The data in table 3 show that the mean liver weights of all groups of female animals and of those of males on diets containing 400 p.p.m. DDT, or more, were significantly larger than the corresponding controls. The kidneys and spleens showed less striking effects than the livers; however, the differences for the kidneys of all groups on 600 and 800 p.p.m. DDT were signif-

icant; the differences in the mean weight of the spleens were not significant. The hypertrophic effects of DDT become more striking when it is remembered that the lighter organs came from the heavier control animals, and the heavier organs from the lighter experimental animals. We have shown in another experiment from this laboratory (7) that this hypertrophy is not a hydration but an actual increase in the amount of tissue as shown on a dry basis.

Pathology. The findings in the first ten rats dying during the course of the first experiment have already been published (13) as part of a study of the histopathological changes following administration of DDT to 9 species of animals. The 38 rats discussed in that paper had been treated for periods ranging from a few days to 32 weeks. In general, those subjects treated for the longer periods showed changes of the type about to be described, while with shorter periods of treatment the affected animals showed toxic lesions of a more acute and nonspecific nature.

Of the 60 rats started on the first experiment, 47 were sent to the pathology laboratory for examination, and of this latter number, 36 were examined microscopically as outlined in the next paragraph for the rats of the second experiment, with minor exceptions. Since the second experiment involved a much larger number of animals, and since the findings in the two series were essentially similar, the principal portion of the description will refer to the findings in the second series, with mention of any difference in the first series when such a difference was present. Of the 168 rats started on the second experiment, 145 were sent to the pathology laboratory for examination, and of this latter number 126 were examined microscopically in a uniform manner. Advanced post mortem autolysis accounted in the majority of instances for the difference between the number in each of the last two categories and the number in the preceding one.

Paraffin-embedded sections stained with hematoxylin and eosin were routinely made of lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidney, adrenal, testis, thyroid and (except in the 200 p.p.m. and control groups) hind leg muscles. Ovary and uterus were sectioned in about half the females, and parathyroids were encountered in about half the thyroid sections. Other structures such as lymph nodes, hing leg bones, and bone marrow, were sectioned in a moderate number of instances, about two dozen of each. Special stains for fat and for iron-containing pigment were done in a few instances.

Perhaps the one outstanding gross change in the treated animals was the increased size of the liver, as shown in table 3. In about a fourth of the animals the liver had a "nutmeg" appearance, more frequent on the higher than on the lower dosage levels, and not seen in the controls. Five rats, three in the 800 p.p.m. in oil group, had a yellowish or tan tinge to the liver. In 4 rats, scattered among the various dosage levels, the adrenals were specifically noted to be large. Eight animals, 4 receiving 800 p.p.m. in oil, had bloody material in the stomach or small intestine. The gross characteristics concerned in the slight tendency of DDT to produce liver tumors will be dealt with later in this summary. The

external appearance of the rats, and the lungs, heart, spleen, lymph nodes, pancreas, kidneys, testes, uterus, ovaries, thyroid and parathyroids, showed no gross effects from DDT, except for weight reduction in some instances.

Microscopically, the observations led to the same conclusion as was found in the first two-year experiment, namely, that the chief lesion in long-term experimental rats is a moderate degree of liver damage of a characteristic type. Also noted in both series was a minimal hepatocarcinogenic tendency, evident late in the experimental period. A finding not noted in the first series, because it contained no females, was a stromal fibrosis and cellular proliferation in the ovary; this, like the tumorigenic tendency, occurred only after about the ninetieth week of feeding. Fatty change in the liver, although not of high grade, was more evident in the second series. Also evident microscopically were a slight generalized increase in size of the adrenals, a slight generalized increase in number of interstitial cells in the testis, and a slight brown pigmentation of the epithelium of the renal convoluted tubules. The latter three changes were all of minor degree, but some of them are perhaps of physiological interest.

The characteristic microscopic change in the liver was proportional to dosage level, although the lower grades of the change were generally present even at the lowest dosage level of 200 p.p.m., and in the first series at 100 p.p.m. No difference in intensity could be observed between dry and oily dosage forms at the same dose levels, or between sexes. The lesion consisted principally in hypertropy and increased cytoplasmic oxyphilia of the centrolobular hepatic cells, plus increased basophilia and margination of the cytoplasmic granules, and a tendency to hyalinization of the remainder of the cytoplasm. This general type of change in the liver has been noted by us (13) in animals other than the rat (mice, rabbits) given DDT, and also in at least one other laboratory (14) in animals receiving DDT. In a small proportion of the rats the typical change appeared to have migrated peripherally in the lobule, or to have been overshadowed by other changes, principally necrosis. The periportal hepatic cells, only rarely showing the characteristic DDT changes, were generally somewhat atrophic. The increased weight of the livers of the treated animals could easily be explained on a basis of centrolobular cellular hypertrophy, when it is considered that doubling each dimension of the hepatic cell would increase its volume approximately eight times; an increase in the number of hepatic cells would not have to be invoked.

Centrolobular necrosis, or focal necrosis within centrolobular areas, both superimposed on the typical DDT change, occurred in many of the livers of the treated rats. The same condition was seen to a lesser extent in the first series. It was somewhat less frequent at the lower dosage levels, and was absent in the controls. It was rather distinctly more frequent in those animals found dead than in those surviving the experimental period. The necrosis generally had an acute appearance and it seems reasonable to assume that much of the necrosis was a terminal phenomenon in dying animals, occasioned by the release of DDT from storage in body fat (7) when such fat was used up during the terminal period of semi-starvation. Vacuolation of the hepatic cells in the paraffin sections,

interpreted as evidence of fatty degeneration, was noted in 13 of the treated rats and in one control. It was generally of slight degree.

Tendency to hepatic tumor formation was, on the basis of comparison with many hundreds of rats of similar age, definite but minimal in both two-year series. Altogether, in both experiments, 4 rats each had one or more small hepatic cell tumors, from 5 to 12 mm. in diameter, paler than the surrounding liver tissue on gross examination, not sharply circumscribed microscopically, and composed of cells larger than those in the rest of the liver. Lobular architecture was almost obliterated. Mitoses were not noted. Some cells had foamy cytoplasm; some cells showed DDT changes of a degree greater than that elsewhere. Tumors of this type are not a sharply defined entity, and the question of their nomenclature cannot be treated here. They would probably be generally called ademonas because of their relative size, discrete gross appearance, and almost total loss of lobular architecture. There might be almost as much justification for considering them low grade hepatic cell carcinomas.

Eleven other rats showed varying amounts of nodular adenomatoid hyperplasia; the nodules were generally of 1 to 3 mm. diameter, and were usually noted grossly as scattered yellowish foci. Nodules smaller or less distinct microscopically were not diagnosed as adenomatoid hyperplasia. The microscopic appearance was essentially the same as in the larger tumor masses; difference in size is chiefly responsible for the difference in terminology. Nodular adenomatoid hyperplasia is almost never seen in our rat livers except after treatment with a few distinctly tumorigenic substances. About 1% of our older rats will spontaneously show distinct hepatic cell tumors, usually 1 to 2 cm. in diameter. The exact incidence has not yet been calculated, but it appears to be 1% plus or minus a few tenths of one per cent, there being about a dozen tumors in as many hundred rats over 18 months of age. By chance, then, one or at the most two tumors in the liver might be expected in the 75 or so rats fed DDT for 18 months or more. Taken together, the 15 rats having either liver tumor or nodular adenomatoid hyperplasia are numerically enough to strongly suggest a distinct although minimal tumorigenic tendency of DDT. All 15 rats had survived 84 or more weeks of the experimental period. This age distribution is essentially the same as in those rats with spontaneously occurring liver tumors, or in those developing tumors after the administration of selenium in the diet (15).

Increased size of the adrenal, and an increased number of interstitial cells in the testis, were changes which were generally minimal enough in degree so that in an individual instance a diagnosis of abnormality would not be warranted; however, they were frequent in occurrence, and in a few instances were great enough in degree to be distinctly seen. The renal tubular pigmentation, wherein clumped masses of brown non-ferrous pigment were present in generally small quantity in the epithelial cells of the convoluted tubules, was noted in 9 scattered rats among the treated groups of the second series, and not in the first series. This change is of minor significance and has been noted after treatment with several substances other than DDT.

Nearly all ovaries in rats surviving 89 or more weeks of the experimental period

showed a peculiar change, present at all dosage levels and not in the controls, in fact not noted previously in any of our rats. The change consisted in an actual as well as a relative increase in amount of stroma, with the presence within this stroma of rather numerous relatively large, generally irregular, pale cells with a moderate amount of cytoplasm. Some of the cells were arranged in glandlike formations. So little has been written on the pathology of the senile rat ovary that the significance of these changes is uncertain. Both treated and control rats showed decreased numbers of follicles, which is to be expected as a senile change.

Focal necrosis of voluntary muscle (hind leg muscles were sectioned) was generally of minimal or questionable degree, but was distinct in a few instances at 600 or 800 p.p.m. A high grade of this lesion had not been expected, but since voluntary muscle had been, next to liver, the structure most damaged by DDT in more acute experiments, its examination was desired in this one as an additional factor of evaluation between dose levels. It is very difficult to determine a minimal degree of old muscle damage (after replacement with scar tissue) with certainty; however, even a minimal amount of acute necrosis is easy to see. Some degree of change was noted in one-fourth to one-half the rats on 600 and 800 p.p.m., and curiously enough, more frequently at the lower of these levels than at the higher. No difference between dosage forms could be distinguished. At 400 p.p.m., only 2 rats in 21 showed changes and these were of ± degree. Since this is about the expected incidence in untreated rats, muscles in the 200 p.p.m. and control groups were not examined routinely.

Certain minor changes were best seen on the higher dosage levels. Splenic atrophy and reduced numbers of secretory granules in the acinar cells of the pancreas were accompaniments of inanition. A slight degree of testicular atrophy may also belong here, or it may be a direct toxic effect of the DDT. While some spleens were atrophic, other were slightly hyperplastic, suggestively a DDT effect; there was also a suggestion of minimal thyroid hyperplasia. Bone marrow was sectioned in 21 treated rats and, as in the thyroid, there was suggestive minimal hyperplasia at the higher dose levels. The bleeding into the gastrointestinal tract mentioned under the gross findings came from small foci of hemorrhagic necrosis in the mucosa of the glandular part of the stomach; this is not specific for DDT.

Bile duct proliferation, splenic and adrenal pigmentation, small ulcers in the forestomach (proventriculus), renal tubular atrophy, protein material in renal tubules, focal thyroid hyperplasia, islands of Langerhans, lymph nodes, heart, lung, stomach, small intestine, colon, uterus, parathyroids, and hind leg bones were the same in the DDT-treated groups as in the controls and in the general run of our comparable rats. Lymphosarcomas of the lung were less frequent in the second series than in the first, but this fact has probably no relation to DDT. Other tumors, and leukemia, showed the usual incidence.

PART II. LIVER RESTORATION IN RATS FED DIETS CONTAINING DDT FOR 12 WEEKS. A group of 16 female rats were given 1000 p.p.m. DDT in the diet for

12 weeks. At this time 4 rats were sacrificed, and the remainder was sacrificed in pairs after placing them on the same diet without DDT for additional periods of 1, 2, 4, 6, 8 and 10 weeks respectively. In the 4 rats sacrificed at the termination of feeding, the histopathological changes were typical of DDT poisoning. Changes were almost entirely confined to the liver, in which there was an enlargement of the centrolobular hepatic cells, with more oxyphilic cytoplasm and more distinct large basophilic granules than in the slightly atrophic cells of the peripheral halves of the lobules. There was a slight tendency to cytoplasmic hyalinization and peripheral segmentation of the basophilic granules in the centrolobular cells. The thyroid showed slight colloid depletion and the accumulation of debris within follicles. Leg muscle, heart, lung, spleen, pancreas, gastrointestinal tract, kidney, adrenal, bone and bone marrow were negative.

In the rats sacrificed at intervals after cessation of feeding DDT, those sacrificed at 1 and 2 weeks afterwards showed livers very similar to those described in the preceding paragraph, while at 4 weeks and 6 weeks there was little of this change, and the livers could have been passed as showing no distinct damage, had not residues of a specific nature been sought. The livers at 8 and 10 weeks had a normal appearance.

Discussion. The observations of this experiment show that chronic poisoning with small amounts of DDT is characterized by degenerative changes in the liver and other organs. This toxicity places a definite and inherent danger in the consumption of small amounts of DDT for a long time. The storage and the slow excretion of DDT increase the possibility of chronic poisoning. Another characteristic is the wide variation in individual susceptibility within the same species as well as between different species of animals (3). In order to correlate the dosage levels with the body weights of animals, the intake of DDT was calculated in terms of the daily intake per kgm. of body weight (chart 1). These values were calculated from the weekly food intake of the rats from both experiments at the various monthly intervals. The DDT intake of the rats at any given dosage level decreased rapidly during the first 2 months and then became almost constant as shown by the straight line (chart 1) at about 6 months on the experimental diet. This fact is accounted for by the change in the growth rate of the rats from the fast growing period to the plateau period, while at the same time their daily food intake remained almost constant. As noted above, surviving rats on concentrations of 600 and 800 p.p.m. DDT recovered from severe tremors after they had reached the plateau period. The chart shows that the daily intake of DDT in mgm. per kgm. of body weight varied from 52.5 and 73.3 at 1 month to 27.8 and 3.82 at 6 months for the male rats on 600 and 800 p.p.m., respectively. Similarly females ingested from 57.1 and 78.9 to 33.2 and 43.1 mgm. DDT per kgm. of body weight during the same time. Therefore, this early toxicity was produced at least in part by the increased intake of DDT during the first 6 months of the experimental period. Furthermore, part, if not all, of the increased toxicity observed in the female rats was undoubtedly caused by the greater amount of DDT per kgm. of body weight consumed by them than by the male

rats fed a similar concentration. For example, female rats on the concentration of 200 p.p.m. DDT consumed about 10.5 mgm. per kgm. of body weight per day for the greater part of the experiment, while male rats consumed 8.5 mgm. per kgm. of body weight per day for the same period.

As shown by the chart the dosage level of 100 p.p.m. DDT corresponds to about 4.5 mgm. per kgm. of body weight per day for the rat over the greater part of the 2-year experiment. The 100 p.p.m. concentration in the diet appears to be a dosage level of DDT at which only slight chronic poisoning occurs in all

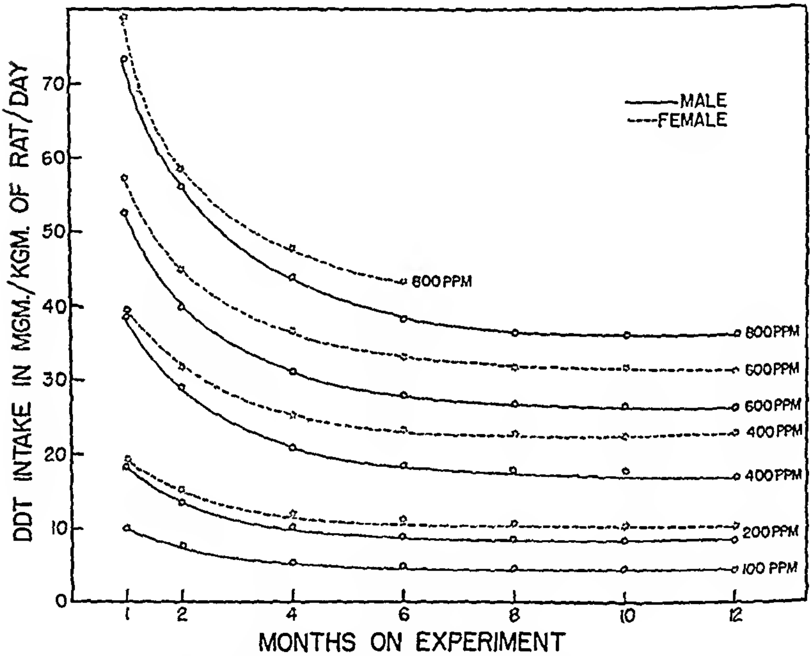

CHART 1. CALCULATED DDT INTAKE IN MGM./KGM. OF BODY WEIGHT IN RATS RECEIVING VARIOUS LEVELS OF DDT IN THE DIET

rats. Another experiment with DDT in dosage levels of less than 100 p.p.m will be reported later.

SUMMARY

In rats fed diets containing from 100 to 800 p.p.m. DDT for a period of 2 years, the following effects were noted.

1. DDT produced chronic toxicity in rats at all concentrations.

2. The outstanding and characteristic histopathological lesion caused by DDT under the conditions of this experiment was a hypertrophy of the centrolobular hepatic cells with an increased cytoplasmic oxyphilia, plus increased basophilia

and margination of the cytoplasmic granules, and a tendency to hyalinization of the remainder of the cytoplasm. Frequently there was superimposed more or less of centrolobular hepatic cell necrosis, much of which had an appearance of being recent.

3. Slight focal necrosis of hind leg muscles was found frequently. This was a much less prominent lesion than liver damage.

4. DDT showed a minimal tendency to cause formation of hepatic cell tumors. This tendency did not operate until after 18 months of feeding.

5. In the later months of the experimental period the ovarian stroma underwent fibrosis and cellular proliferation.

6. The microscopic lesions observed, except for focal necrosis of the hind leg muscles, showed a fairly distinct gradation with dosage level. They varied from slight at 100 p.p.m. to marked at 800 p.p.m. DDT.

7. An increased intake of DDT-containing diet per kgm. of body weight during the fast growing period of the rat produced an increase in toxicity.

8. The greater intake of DDT per kgm. of body weight by female rats than that by male rats on similar concentrations produced an increased toxicity in the females.

9. At concentrations of 400 to 800 p.p.m. DDT, rats showed characteristic nervous symptoms of poisoning. Lower dosages produced an increased irritability. Muscle tremors were more pronounced in female rats than males.

10. Concentrations of 400 to 800 p.p.m. DDT in the diet retarded growth of female rats. In male rats only 800 p.p.m. DDT retarded growth.

11. The livers, and to a lesser extent the kidneys of experimental animals, were larger than those of the controls. These differences were more pronounced in the groups on 600 and 800 p.p.m. DDT.

12. At concentrations of 400 to 800 p.p.m. DDT in the diet, female rats showed an increased mortality rate. DDT at concentrations used in this experiment did not produce any effect on mortality rate of male rats.

13. DDT did not affect the food consumption of rats.

14. The withdrawal of all food from the chronically fed rats on 400 to 800 p.p.m. DDT produced characteristic tremors within 24 hours.

15. No difference in incidence or degree of changes occurred between animals given DDT in dry form and those given DDT in corn oil solutions.

In rats sacrificed at intervals after cessation of feeding 1000 p.p.m. DDT in the diet for 12 weeks, those sacrificed at 1 and 2 weeks afterwards showed damaged livers, while at 4 and 6 there was little change. The livers of rats sacrificed after withdrawal of DDT for 8 and 10 weeks exhibited a normal appearance.

REFERENCES

1. Smith, M. I., and Stohlman, E. F.: Pub. Health Rep., 59: 984, 1944.
2. Woodard, G., Nelson, A. A., and Calvery, H. O.: This Journal, 82: 152, 1944.
3. Draize, J. H., Woodard, G., Fitzhugh, O. G., Nelson, A. A., Smith, R. B., Jr., and Calvery, H. O.: Chem. and Eng. News, 22: 1503, 1944.
4. Neal, P. A., von Oettingen, W. F., Dunn, R. C., and Sharpless, N. E.: Pub. Health Rep. Supplement No. 183, 1944.

5. Philips, F. S., and Gilman, A.: This Journal, 86: 213, 1946.
6. Bing, R. J., McNamara, B., Hopkins, F. H.: Bull. Johns Hopkins Hosp., 78: 308, 1946.
7. Laug, E. P., and Fitzhugh, O. G.: This Journal, 87: 18, 1946.
8. Smith, M. I., and Stohlman, E. F.: Pub. Health Rep., 60: 289, 1945.
9. Ludewig, S., and Chanutin, A.: Proc. Soc. Exp. Biol. and Med., 62: 20, 1946.
10. Telford, H. S., and Guthrie, J. E.: Science, 102: 647, 1945.
11. Woodard, G., Ofner, R. R., and Montgomery, C. M.: Science, 102: 177, 1945.
12. Fisher, R. A., and Yates, F.: Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd. Edinburgh, 1938.
13. Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. G., Smith, R. B., Jr., and Calvery, H. O.: Pub. Health Rep., 59: 1009, 1944.
14. Lillie, R. D., and Smith, M. I.: Pub. Health Rep., 59: 979, 1944.
15. Nelson, A. A., Fitzhugh, O. G., and Calvery, H. O.: Cancer Res., 3: 230, 1943.

PHARMACODYNAMIC STUDIES OF A NEW ANTIHISTAMINIC AGENT, PYRIBENZAMINE (N,N-DIMETHYL-N′-BENZYL-N′-(α-PYRIDYL)-ETHYLENE DIAMINE HYDROCHLORIDE)

II. Effects on Smooth Muscle of the Guinea Pig and Dog Lung

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Received for publication September 24, 1946

In previous communications (1) the antihistaminic and antianaphylactic properties of N,N-dimethyl-N′-benzyl-N′-(α-pyridyl)-ethylene diamine hydrochloride (Pyribenzamine) were demonstrated. Important tests of such properties concern the drug's effects on smooth muscle, notably that of the bronchial apparatus as demonstrated by Mayer (1) in his studies on histamine-induced asthma and anaphylaxis in vivo. We chose other approaches to this problem and this report deals with such studies in the lungs of normal and sensitized guinea pigs and dogs.

A. Studies of the guinea pig lung. *Method.* Lungs of normal and "horse-serum sensitized" guinea pigs of varied ages were prepared according to the modified method of Tainter, Pedden and James (2) in which the lung was continuously perfused with a spscially prepared, aërated fluid at a temperature of 37.5°C. Perfusate was measured at regular intervals and modifications of fluid output by the lung were effected by intratracheal injections of drugs to be studied. After an interval of approximately 15 minutes for control purposes, the following drugs were instilled into the perfusion line just above the suspended trachea: Histamine phosphate, 50 micrograms; Pyribenzamine, 25 to 50 micrograms; Horse serum (Lederle), and in a few experiments, N′-phenyl-N′-benzyl-N-dimethylethylene diamine HCl, known as Antergan (3), for the purpose of comparing this antihistaminic with Pyribenzamine.

Several guinea pigs were sensitized to horse serum some 15 to 20 days previously by injections of 0.5 cc. intramuscularly. Some of these animals were given Pyribenzamine subcutaneously at varying intervals prior to lung excision.

Results. The bronchial tonus of lungs excised from 41 normal and 14 "sensitized" guinea pigs showed no significant deviation from normal even after 3 to 4 hours of non-medicated perfusion. The age of the animals made no difference in responses obtained.

The standard dose of 50 micrograms of histamine consistently produced a marked and immediate constriction of the bronchial musculature as reflected by the great diminution in perfusate per minute (fig. 1). This histaminic contraction was decidedly reduced by 25 to 50 micrograms of Pyribenzamine (fig. 1). These amounts of Pyribenzamine were without marked effect on normal bronchial tonus; however, slight evanescent constriction occasionally followed such ad-

ministration. Also, there was no accumulation of such effect as a result of repetition of these doses of Pyribenzamine.

The protection afforded by Pyribenzamine against histamine-induced contraction of bronchiolar musculature lasted for approximately 20 to 40 minutes but each successive injection of histamine became more effective until complete re-

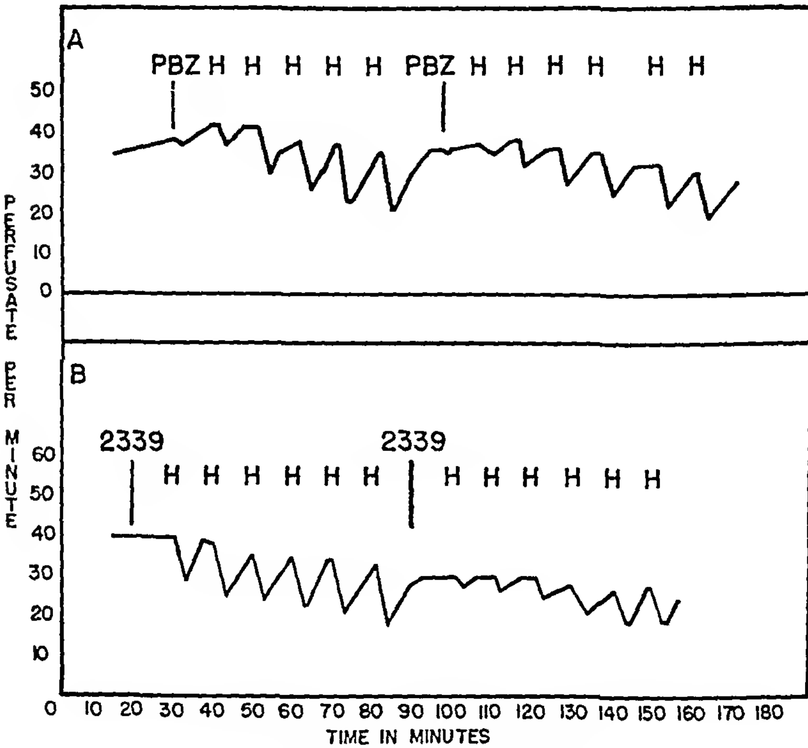

FIG. 1. PERFUSED LUNG OF GUINEA PIG

The rate of perfusion expressed in milliliters per minute is shown on the ordinate and the time in ten minute intervals on the abscissa. The protective effect of Pyribenzamine (PBZ) 0.025 mg. against histamine phosphate, 0.050 mg. is indicated in A; that of Antergan against histamine phosphate in corresponding amounts in B.

covery from Pyribenzamine ensued (fig. 1-A). Simultaneous injection of Pyribenzamine and histamine afforded much less protection than was obtained when Pyribenzamine had been administered some 10 minutes before histamine.

The injection of Pyribenzamine at the time of maximum bronchial constriction seemed to prolong significantly the protective effect of this drug against successive histamine injections (fig. 2). Perfusion of the lung with a solution containing

0.1γ/cc. of Pyribenzamine gave complete protection against the constriction caused by 100 micrograms of histamine.[1]

Approximately equal protection was afforded against histamine-induced contraction by Antergan (RP2339) in all of eleven experiments conducted (fig. 1-B).

Horse serum did not alter the rate of perfusion (fig. 3-a) in the normal lung.

Horse serum caused an immediate and marked bronchiolar contraction in the lung of the guinea pig sensitized to horse serum (fig. 3-b). This lung did not usually recover from such contraction, whereas the normal lung constricted by histamine invariably did (fig. 1).

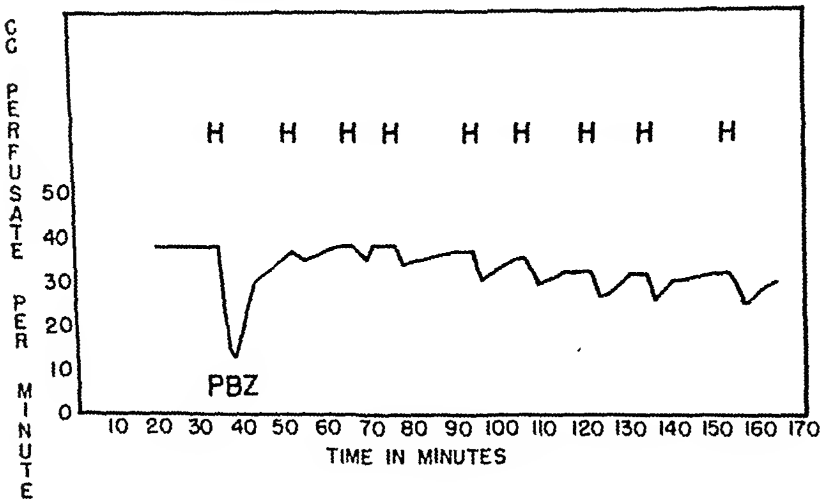

FIG. 2. PERFUSED LUNG OF GUINEA PIG

The rate of perfusion expressed in milliliters per minute is shown on the ordinate and the time in ten minute intervals on the abscissa. The protective effect of Pyribenzamine (PBZ) 0.025 mg. against histamine phosphate, 0.050 mg. is indicated. It is obvious that the injection of Pyribenzamine (PBZ) at the time of maximal constriction caused by histamine has markedly enhanced the antihistaminic action of Pyribenzamine (PBZ).

The bronchiolar contraction produced by horse serum in the sensitized lung in vitro was not favorably affected by Pyribenzamine (fig. 3-c).

In the perfused lungs of 7 guinea pigs to which Pyribenzamine, 20 mg./kg. had been given subcutaneously 20 minutes before excision of the lungs, the antihistaminic agent had some effect in three, but no effect in four (fig. 4).

The blue dye, T-1824, was employed in several experiments in order to determine the distribution of the perfusing fluid and its rate of perfusion; the results and their significance have been reserved for discussion below.

Discussion. The protection which Pyribenzamine afforded the bronchial musculature of the guinea pig against histamine in this investigation confirmed

[1] We are indebted for these findings to our associate, Dr. B. N. Craver.

such protection which Mayer, Huttrer and Scholz (4) had demonstrated for this compound in vivo in mice and which Loew, Kaizer and Moore (5) had shown for the benzhydryl alkamine ethers of Rievschel (6) against histamine-induced asthma in intact guinea pigs.

Although the antihistaminic effect in vivo (4) was often complete and prolonged with adequate dosage of Pyribenzamine, the drug in our in vitro experiments was slightly less effective. In an attempt to prolong the effect in vitro, Pyribenzamine was given at the height of bronchial constriction so that the attending limited output with slower rates of perfusion might permit longer contact

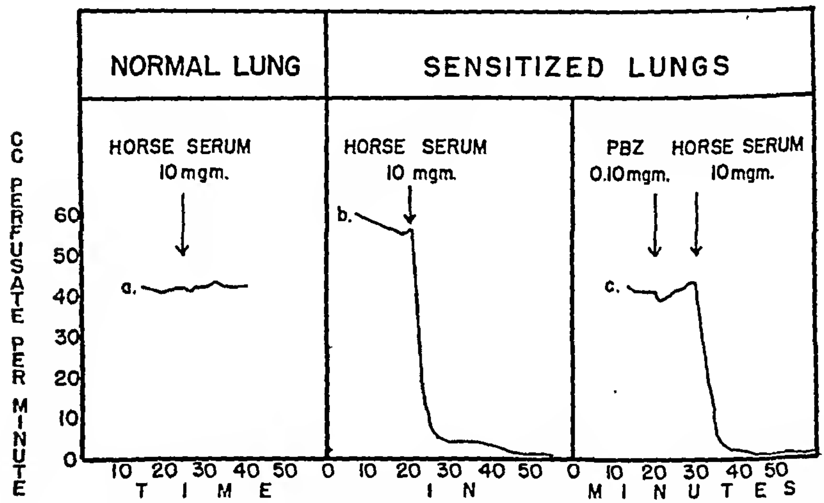

FIG. 3. STUDIES OF THE PERFUSED, EXCISED GUINEA PIG LUNG IN ANAPHYLAXIS. PERFUSED LUNGS OF NORMAL GUINEA PIG AND OF GUINEA PIGS SENSITIZED TO HORSE SERUM

The rate of perfusion expressed in milliliters per minute is shown on the ordinate and the time in ten minute intervals on the abscissa. The lack of effect of 10 mg. of horse serum in the normal lung is shown in a. The retarding effect (bronchospasm) of 10 mg. of horse serum on the perfusion in the lung of the sensitized guinea pig is seen in b and the failure of 0.100 mg. of Pyribenzamine (PBZ) to protect against such retarding effects in c.

of the antihistaminic agent with the receptive substance in the bronchial musculature. Definite advantage was gained (fig. 2) by this technique and sufficient encouragement was afforded to warrant the assumption that continuous perfusion of Pyribenzamine might offer continued protection against histamine, thus more nearly approximating those conditions prevailing in vivo, i.e., rather constant medication for longer intervals. Our results indicate that this assumption was justified.

It is difficult to interpret the lack of protection by Pyribenzamine against horse serum anaphylaxis in vitro, since this is in direct contrast to results with anaphylaxis in the intact guinea pig (4). It may be that bronchial constrictors

other than histamine attend such reactions when the anaphylactogen is applied; slight protection was offered in vitro, however, to some lungs of the sensitized animals receiving Pyribenzamine subcutaneously prior to perfusion of the lung. This protection may have been based on the specific antihistaminic property of Pyribenzamine. On the other hand, the lungs of the remaining sensitized pigs received no protection against the antigen in vitro, even though sufficient amounts of Pyribenzamine had been given in vivo to produce marked toxic manifestations such as tremors, gasping and incoordinated locomotion. Such animals may require continued medication with well tolerated amounts of Pyribenzamine

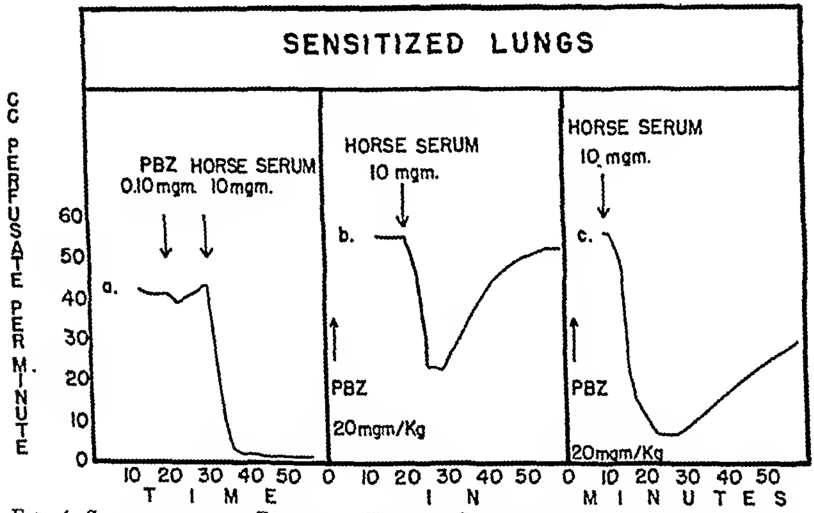

FIG. 4. STUDIES OF THE PERFUSED, EXCISED GUINEA PIG LUNG IN ANAPHYLAXIS
PERFUSED LUNGS OF SENSITIZED GUINEA PIGS

The rate of perfusion expressed in milliliters per minute is shown on the ordinate and the time in ten minute intervals on the abscissa. The failure of 0.100 mg. of Pyribenzamine [illegible] 20 mg./kg. subcutaneously; this partial protective action is represented by recovery from such retarding effects rather than by complete protection against it.

for hours, or even days, in order to effect a tissue concentration of the drug adequate for protection against the anaphylactogen administered in vitro. Why the sensitized animal in its entirety can be protected in vivo while its bronchial musculature in vitro does not lend itself to such protection is a moot point. The alternative possibility is that the lung, although chiefly concerned, may not be the only important organ entering into the anphylactic sequence in the guinea pig; there may be other organs or systems receiving protection from Pyribenzamine in vivo which are not demonstrable in the experiment with the isolated, perfused lung.

The interpretation placed upon our results seems valid in the light of results gained with the blue dye, T-1824; apparently, Pyribenzamine and histamine permeated all parts of the lung since the blue dye, injected intratracheally, diffused through the perfusate into the entire lung bed. It is thus presumed that the responses of the bronchial muscle to all drugs were "in toto" reactions rather than variable, regional ones. The consistency of results obtained with control injections of histamine from lung to lung would tend further to substantiate the conclusions drawn from all antihistaminic reactions observed with this technique.

B. STUDIES OF THE DOG LUNG *Method.* The bronchial tonus of the lungs of dogs whose chests were surgically prepared under Sodium Pentobarbital for the insertion of the Jackson (7) chest plethysmograph was registered tambourically. Artificial respiration was produced with the Harvard Respirator Pump and blood pressures were recorded concomitantly with the Anderson (8) glass membrane manometer.

Skeletal muscle tone was insulated against central control by dihydrobetaerythroidin (9) and it was customary in most of our experiments to section the phrenic, and usually the vagal nerves, prior to the administration of other drugs. These drugs included histamine phosphate, Pyribenzamine hydrochloride, Mecholyl hydrochloride, physostigmine salicylate and atropine sulphate; all were administered intravenously on a basis of milligrams per kilogram of body weight. Several dogs which were sensitized 3 to 4 weeks previously to horse serum received this antigen intravenously.

Results. The bronchial muscle of the dog was frequently, but not at all consistently, contracted by histamine in varying dosage. The usual doses employed in 20 dogs in this study ranged from 15 to 50 micrograms and if once effective, successive injections quite consistently duplicated this constriction (figs. 5 and 6). Physostigmine was occasionally employed with the hope of rendering more responsive the bronchial muscle of those dogs failing to respond to histamine, but the latter drug's action was not enhanced by physostigmine given before or after Pyribenzamine (fig. 5).

Histamine-induced bronchial constriction could be completely prevented by Pyribenzamine in sufficient dosage, usually 3 mg., and this protection invariably endured for the rest of the experiment, i.e., for three hours or more (figs. 5 and 6).

Accompanying the bronchial constriction by histamine, there was invariably a marked, acute hypotension, the duration of which was determined by the amount of histamine administered. Fortunately, this hypotension endured for only a few minutes but with successive doses of histamine, normal tension gradually declined in some cases to mean levels 30% to 50% below normal (fig. 6).

These pressure changes, both acute and gradual, could be combatted to some extent in most instances by Pyribenzamine in 3 mg. dosage (fig. 6). Whereas acute histamine-hypotension might be less marked immediately following Pyribenzamine, it became more marked during the gradual recovery of average pressure to normal levels (figs. 5 and 6).

In none of ten experiments with sensitized dogs were we able to demonstrate any contraction of the bronchial musculature after administration of horse serum (fig. 7), although anaphylaxis was evident from the marked hypotension attending such administration of serum. In this type of experiment, however, the

bronchi still could react strongly to histamine, either before or after horse serum. The vascular anaphylactic reaction could be prevented by Pyribenzamine in adequate dosage, usually 3.5 mg. (1-d).

Acetylcholine or Mecholyl, with physostigmine, invariably produced hista-

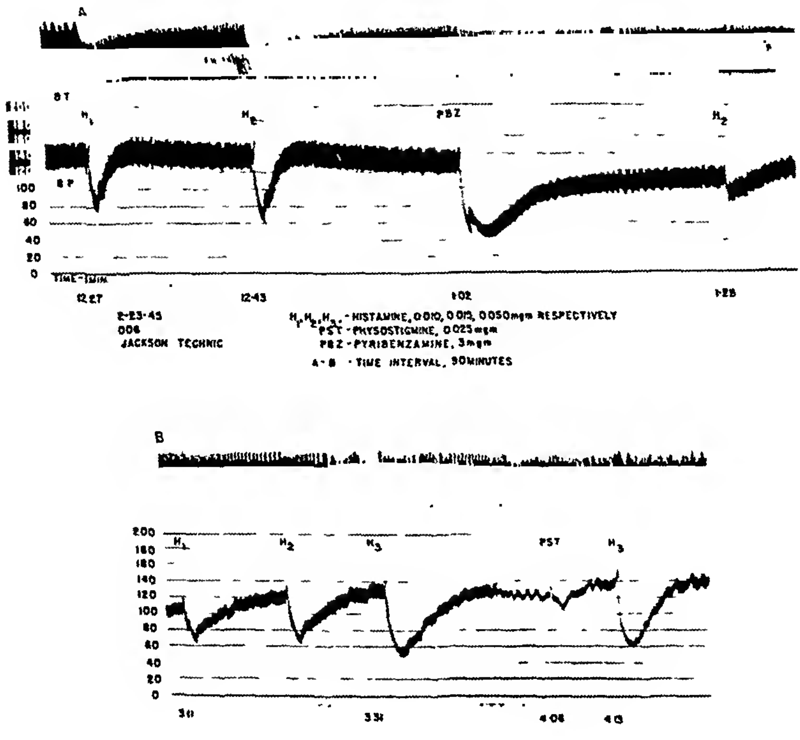

FIG. 5. 2-23-45. Dog, 10 kg., pentobarbital anesthesia, Jackson plethysmograph. From above downward: bronchial tonus, arterial pressure, time in minutes. The protective effect of Pyribenzamine, 3 mg. against bronchial constriction and hypotension due to histamine, 15 micrograms, is evident. Attempts to break through this anthistaminic block by sensitizing receptor substances to histamine with physostigmine were unsuccessful, even when 50 micrograms of histamine were employed. Between A and B a time interval of approximately 90 minutes elapsed, during which vagal faradization, histamine, 15 micrograms, and dihydrobetaerythroidin, 5 mg., were exercised.

mine-like reactions in normal and sensitized animals in relation to both bronchial constriction and hypotension. Pyribenzamine was of little value, prophylactically or correctively, in these situations; atropine was of greater value (fig. 8).

Discussion. The failure of the bronchial musculature to respond antigenically to horse serum by contracting was not too surprising since in the dog, as is well

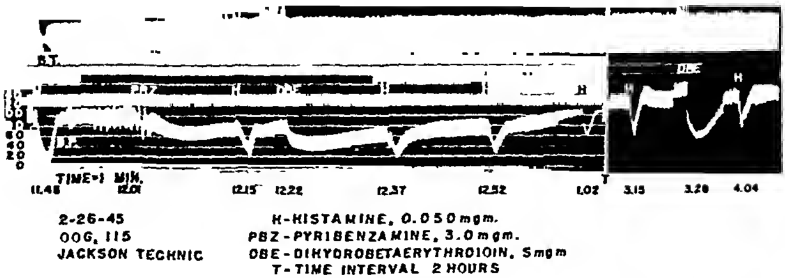

FIG. 6. 2-26-45. Dog, 12.9 kg., pentobarbital anesthesia, Jackson plethysmograph. From above downward: bronchial tonus, arterial pressure, time in five second intervals. The protective effect of Pyribenzamine, 3 mg., against bronchial constriction and hypotension due to histamine, 50 micrograms, was evident, even after four hours. Blood pressure responses were approaching normal at this time but the bronchial smooth muscle was still completely protected against histamine constriction. Mean blood pressure was also belatedly elevated following Pyribenzamine, even in the presence of several doses of dihydrobetaerythroidin HCl, 5 mg., and histamine. Between A and B time interval of approximately two hours elapsed during which six doses of histamine, 50 micrograms, and two doses of dihydrobetaerythroidin, 5 mg., were exercised.

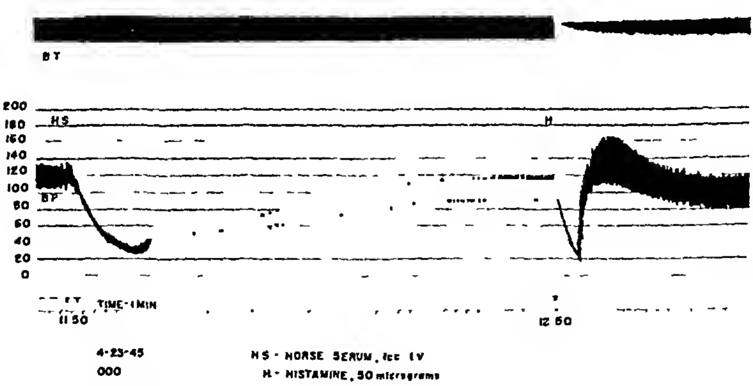

FIG. 7. 4-23-45. Dog, 14.3 kg., sensitized to horse serum, 1.5 cc. intramuscularly on 4-5-45. Pentobarbital anesthesia, Jackson plethysmograph. From above downward: bronchial tonus, arterial pressure, time in minutes. Horse serum, 1 cc. injected intravenously produced acute, marked hypotension which returned almost to normal in approximately 25 minutes. No bronchial constriction attended this anaphylactic reaction, but histamine phosphate, 50 micrograms, produced intense constriction of the bronchioles and simultaneous acute and severe hypotension.

known, the liver seems to be the organ chiefly affected in anaphylaxis (10). If histamine were released in abundance in the anaphylactic state, it could be

anticipated that a sufficient amount of this imidazole might slip by the "hepatic hurdle" to effect at least a slight but appreciable contraction of the bronchial musculature so as to be registered kymographically; but such was not the case, despite the fact that very significant contractions had been normally obtained

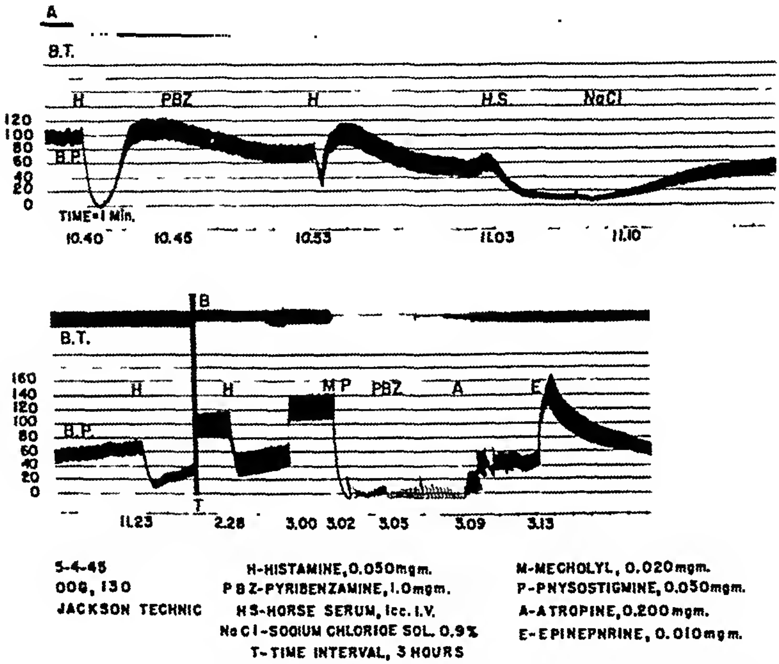

Fig. 8. 5-4-45. Dog, 10.1 kg., sensitized to horse serum, 5 cc., intravenously and 5 cc. intramuscularly on 4-13-45. Pentobarbital anesthesia. Jackson plethysmograph. From above downward: bronchial tonus, arterial pressure, time in five second intervals. The bronchial constriction produced by histamine phosphate, 50 micrograms, was inhibited by Pyribenzamine, 1.0 mg., which simultaneously had some inhibitory effects on the acute hypotension produced by histamine and no prophylactic action against horse serum-induced hypotension. Physiologic sodium chloride solution was necessary to restore arterial pressure. Pyribenzamine, intracardially, was without benefit against acute hypotension following Mecholyl, 20 micrograms, and physostigmine, 50 micrograms, whereas, atropine sulphate, 0.200 mg. and epinephrine, 0.010 mg. returned arterial pressure toward normal. Bronchial tonus, increased to maximum by Mecholyl and physostigmine, was only partially relieved by Pyribenzamine but returned to normal with atropine and epinephrine. Between A and B a time interval of approximately three hours elapsed during which 5 doses of histamine, 50 micrograms, and dihydrobetaerythroidin, 3 mg., were exercised.

from histamine administered in only 10 microgram dosage (fig. 5). These facts suggest that the amount of histamine released in the anaphylactic state is either: (a) insufficient or (b) does not arrive in adequate concentration to produce bronchial constriction; a third possibility is that (c) other elements are responsible also

for anaphylaxis in the dog. There may be other hypotensive components present which have no appreciable bronchiospastic effects.

Protection against the acute hypotension of anaphylaxis (1-d) by the anti-histaminic substance, Pyribenzamine, was appreciable and this lends support to the conclusion (11) that histamine is responsible for many of the signs and symptoms attending anaphylaxis. In other words, as has been suggested (4 and 5), therapeutic agents of this type become pharmacologic and diagnostic tools in the sense that they can detect the presence of released histamine in the allergic state by amelioration or prevention of symptoms.

In a previous communication (1-e) it was demonstrated that Pyribenzamine differs from atropine in its capacity to suppress histamine-induced salivation. Evidence of such differences in our present experiments seems to have been afforded by the fact that bronchial constriction and acute hypotension, as produced by acetylcholine, or by Mecholyl with physostigmine, were not suppressed or corrected by Pyribenzamine but by atropine. Hence, Pyribenzamine seemed again to be predominantly quite specifically antihistaminic rather than anti-cholinergic.

The fact that Pyribenzamine was an effective antihistaminic agent in the severe test objects studied warrants clinical usage of this and similar agents in various clinical conditions, notably allergies, in which histamine may play a major role in the drama of varied symptomatology.

The mode of action of Pyribenzamine is still problematic but insulation of the histamine-receptive substance peripherally against this imidazole seems highly probable (1-e, 12) through the proposed mechanism of competitive inhibition.

SUMMARY AND CONCLUSIONS

1. Perfused lungs of normal guinea pigs could be significantly protected against histamine constriction of their bronchioles by Pyribenzamine (N,N-dimethyl-N′-benzyl-N′-(α-pyridyl)-ethylene diamine hydrochloride). Such activity is comparable to that afforded by Antergan (N′-phenyl-N′-benzyl-N-dimethyl-ethylene diamine HCl). Better protection of perfused lungs was gained when Pyribenzamine was constantly perfused throughout the course of the experiment.

2. Although sensitized guinea pigs could be protected by Pyribenzamine in vivo against the anaphylactogen, horse serum, the perfused lungs of such pigs were only slightly, or not at all, afforded such protection against horse serum by Pyribenzamine in vitro.

3. The bronchial musculature of the sensitized dog, as studied by the Jackson technic, was not contracted by the anaphylactogen, horse serum, even though marked anaphylaxis was evident from the accompanying acute hypotension.

4. The bronchial musculature of the sensitized and normal dog was frequently constricted to a marked degree by histamine, and the well-known, marked acute hypotension effected by this drug invariably accompanied such bronchial constriction. The antihistaminic agent, Pyribenzamine, effectively combatted the hypotensive and bronchiolar constricting effects of histamine and offered significant protection against anaphylactic hypotension.

5. The value of such an antihistaminic and antianaphylactic agent in certain clinical situations, particularly allergic states, is evident.

REFERENCES

1. a. Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Science, 102: 93 (July) 1945.
   b. Mayer, R. L.: J. Allergy, 17: 153 (May) 1946.
   c. Mayer, R. L., Hays, H. W., Mathieson, D., Rennick, B., and Yonkman, F. F.: J. Lab. & Clin. Med., 31: 749 (July) 1946.
   d. Yonkman, F. F., Hays, H. W., and Rennick, B.: Feder. Proc., 4: 144, 1945.
   e. Yonkman, F. F., Chess, D., Mathieson, D. and Hansen, N.: This Journal, 87: 256, 1946.
2. Tainter, M. L., Pedden, J. R., and James, M.: This Journal, 51: 371, 1934.
3. DeCourt, P.: Presse Méd., 51: 501, 1943.
4. Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Feder. Proc., 4: 129, 1945.
5. Loew, E. R., Kaiser, M. E., and Moore, V.: This Journal, 83: 120, 1945.
6. Rievschel, G., and Gruhzit, O. M.: Feder. Proc., 4: 150, 1945; and quoted by Loew (5).
7. Jackson, D. E.: Experimental Pharmacology and Materia Medica. 2nd Ed., p. 304, Mosby, 1939.
8. Anderson, F. E.: J. Lab. & Clin. Med., 26: 1520, 1941.
9. Ramirez, E., and Rivero, M. D.: Anales Inst. Biol. (Mex.), 6: 301, 1935.
   Lehman, A. J.: This Journal, 60: 71, 1937.
   Unna, K., Kniazuk, M., and Greslin, J. G.: This Journal, 80: 39, 1944.
10. Dragstedt, C. A.: Physiol. Rev., 21: 563, 1941.
11. Dale, H. H. and Laidlaw, P. P.: Am. J. Physiol., 41: 318, 1910.
    Dale, H. H.: Lancet, 1: 1285, 1929.
    Dragstedt, C. A., and Gebauer-Fuelnegg, Erich: Am. J. Physiol., 102: 512, 1932.
    Gebauer-Fuelnegg, Erich, and Dragstedt, C. A.: Am. J. Physiol., 102: 520, 1932.
    Dragstedt, C. A., and Mead, F. B.: This Journal, 57: 419, 1936.
    Ojers, Gaylord, Holmes, C. A., and Dragstedt, C. A.: This Journal, 73: 33, 1941.
    Code, C. F.: Am. J. Physiol., 127: 78, 1939.
12. Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, C. A.: This Journal, 85: 122, 1945.

A METHOD FOR THE DETERMINATION OF CUTANEOUS PENETRATION OF MERCURY[1]

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Received for publication September 16, 1946

It has been well established that the skin is permeable to mercury. Sufficient quantities of the element may enter the body by this route to cause not only a measurable excretion in the urine, but in some cases even symptoms of mercurialism. The degree to which mercury penetrates the skin, as reflected by urinary excretion of the metal, has been used by a number of investigators (1–7) to study the therapeutic efficiency of mercury compounds, ointment preparations and inunction procedures. Our preliminary experiments, however, indicated that urinary excretion, as a measure of cutaneous penetration of mercury, was not only insufficiently sensitive to discriminate between various types of mercury preparations, but was also time-consuming and subject to inherent contamination errors more difficult to overcome in animal than in human exposures. The following method which proved effective as an assay procedure in our hands was, therefore, developed.

PRINCIPLE. This method substitutes the principle of storage of mercury for excretion of mercury, as a means of measuring how much has penetrated the skin. Of all organs of the body, the kidney has the greatest capacity for storing mercury. This observation is not a new one, and has been reported a number of times (8, 9, 10). Even when minute amounts enter the organism, amounts so small as to escape detection in the urine, storage of mercury in the kidney occurs in quantities sufficiently large for routine microanalytical determination. At any time following exposure to mercury, the amount in the kidney may be assumed to be a balance between three dynamic factors. 1) The rate of entrance of mercury, 2) The rate of excretion of mercury, and 3) The rate of storage of mercury. If the rate of excretion is slow, and the rate of storage rapid, then it becomes possible to use the mercury content of the kidney as a measure of the amount which has penetrated the skin. Obviously this measure is only a relative one, but is chiefly useful in comparing the penetrating abilities of different types of mercury ointments.

PROCEDURE. Rabbits and rats were used for the assay. A clipped area on the back was innuncted for 2 minutes with a mercury ointment, covered with rubber sheeting or celluloid, and contact of the ointment with the skin maintained for 24 hours. At the end of this time the animals were killed with ether, exsanguinated, and liver and kidney removed for an-

[1] A portion of the funds used in this investigation was supplied by a transfer, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Division of Pharmacology of the Food and Drug Administration.

alysis of mercury (11). All results were expressed in micrograms per gram of wet tissue. The average concentration of mercury in the kidney was approximately ten times greater than in the liver; in general the degree of discrimination obtainable with the liver values was much lower. Nevertheless, analysis of the livers for mercury was always carried out, since it served as a convenient independent check of the method. For example, failure of correlation between kidney and liver mercury levels tended to discount the value of an assay. In such a situation it was customary to verify the results either by repetition of the entire assay or by addition of more acceptable analyses. Owing to the natural variability in a group of animals, it was usually necessary to use from 6 to 8 animals on each treatment in order to be able to recognize significant differences between inunction procedures or ointments. The inunction area in the rat was 29 $(cm.)^2$ ($1\frac{1}{2}$ by 3 inches) and in the rabbit 155 $(cm.)^2$ (4 by 6 inches). Taking the average weight of the rat used in these experiments as 250 grams, and that of the rabbit as 2500 grams, each of the areas on the respective species was calculated as 8 per cent of the total body surface. Total body surface in square meters was calculated by Meeh's formula (12): $S = K \sqrt[3]{(\text{Weight})^2}$, where K for the rat was taken as 0.091, and for the rabbit (without ears) as 0.108. The sizes of the respective areas were kept constant and were not adjusted to allow for variations in the body weights of the animals. The compound of mercury used in the preparation of the ointments for this study was calomel. It was suspended in 30 per cent concentration in a number of different vehicles, the composition of which will not be stressed at this time, since this is to be discussed in the following paper. A constant amount of 30 per cent calomel ointment was always applied: 0.4 gram for the rat, 4.0 grams for the rabbit. A considerable excess of the ointment always remained on the skin after inunction.

Stringent precautions are necessary in studies of cutaneous exposure to limit the entrance of mercury into the body to the skin route. Oral contact with the inuncted skin area obviously invalidates any conclusions that can be drawn from the analyses. Since the effective handling of animals to prevent oral contamination is difficult, a brief description of the techniques used in this study will be given.

Because of the greater availability, a larger number of rats than rabbits was used. It was, however, not feasible to keep the rats in individual stocks, and, therefore, a technique for wrapping the animals was developed which proved rapid and effective. The animals were lightly etherized and the hair removed around the trunk between shoulders and hips. Clipping with an electric clipper did not remove the hair completely, but left a fine stubble approximately 0.5 mm. long. This was to be preferred to other methods of depilation which, while more complete, might cause minor skin lesions. The area to be inuncted extended saddle fashion from the back around to each flank, and, in order to prevent spread of the ointment, was delimited with strips of celluloid cemented to the skin. After inunction, and without removal of the excess ointment still remaining on the skin, the animal was wrapped in a piece of celluloid sheeting formed into a cylinder fitted snugly around the trunk. The edges of the cylinder were cemented to the skin at the shoulders and hips so as to prevent slipping of the shield or possible seepage of the ointment. As additional protection, the shield was covered with a layer of adhesive tape extending well beyond its edges. Thus "trussed", the animals were placed in individual cages with access to food and water. Experience with several hundred rats so prepared has shown that the celluloid shield proved to be sufficiently rigid to prevent the animal from getting in oral contact with any part of its trunk, yet did not interfere with walking. Although the animals occasionally struggled, the celluloid skin seals were effective in confining the ointment beneath the shield.

In the case of the rabbits, oral contact with the body was completely prevented by immobilizing the animals in a special head-hip stock which has been described elsewhere (13). The animals were kept in the holder during the inunction with the ointment, and for the 24-hour contact period which followed. Rubberized cloth, wrapped about the trunk and secured with tape, was used as skin covering. The animals were not anesthetized for the inunction and wrapping procedures.

Results. Table 1 shows the concentration of mercury in the tissues following cutaneous exposure to a calomel ointment. It can be seen that by far the greatest is in the kidneys; in fact, mercury concentration in the other tissues is of an entirely different order of magnitude. It is to be observed that in the control animals, traces of mercury may be found, particularly in the kidney. Since, however, this concentration is so low (of the order of 1 per cent of the kidney values of the inuncted animals) its importance as an inherent assay error may be disregarded. Following extension of the exposure period to 48 hours, there is a

TABLE 1

Distribution of mercury in the tissues of the rat following cutaneous application of a 30 per cent calomel ointment

(Micrograms mercury per gram wet tissue)

KIDNEY	LIVER	BLOOD	MUSCLE	LUNG	HEART	TESTES	BRAIN	SPLEEN	STOMACH AND CONTENTS	SMALL INTESTINE AND CONTENTS	BONE
Control											
0.28	0		0.11	0.14			0	0			0.08
0.35	0.11		0	0.10			0.10				0.22
0.28	0.08		0.09	0.11			0	0			0
24-hour exposure											
22.3	1.02	0.60	0.34	1.02	0.50	0.10	0	2.03	0.12	0.46	0.16
21.8	0.60	0.58	0.10	0.86	0.72	0.12	0.10	0.86	0.17	0.38	0.26
25.0	0.79	0.35	0.18	1.07	1.17	0.10	0.16	3.00	0.35	0.38	0.43
36.0	0.78	0.56	0.14	1.61		0.05		1.37	0.19	0.44	0.16
31.4	0.98	1.02	0.10	0.91	0.69	0.14	0.24	2.03	0.22	0.60	0.13
26.6	0.88	0.75	0.12	1.82	0.60	0.11	0.14	2.45	0.21	0.57	0.20
48-hour exposure											
32.3	1.49	0.83	0.07	1.86	1.11	0.22	0.16	2.85	0.19	0.53	0.30
40.5	1.95	1.35	0.26	2.18	0.62	0.16	0.21	2.10	0.25	1.04	0.46
44.3	1.12	1.24	0.08	1.53	0.67	0.11	0.22	0.96		0.48	0.32
34.6	0.75	0.87	0.08	1.14	0.58	0.08	0.30	0.84	0.35	0.38	0.24
39.4	1.11	0.89	0.05	1.48	0.81		0.29	1.48	0.11	0.36	0.35
40.5	1.27	0.75	0.07	1.95	1.11	0.25	0.26	1.41	0.20	0.69	0.13

significant rise in kidney mercury which, however, does not obtain for any of the other tissues except perhaps liver and blood.

In addition to the data showing the concentration of mercury in the tissues, some experiments were conducted in which the percentage distribution of the total amount of mercury which had entered via the skin was determined in kidney, liver, blood, carcass and excreta. In order to ascertain this total amount, the entire body minus the section of skin which had been the portal of entry for mercury was analyzed. In discarding this skin containing gross amounts of mercury remaining after inunction, it was assumed that all of this mercury was

still within the outer skin structures and, therefore, "outside" the body. This assumption may be in slight error because it does not take into account the quantity of mercury within the blood and lymph channels of the skin section, which amounts, properly speaking, are "within" the body. However, analyses of non-inuncted skin sections from other portions of the body indicate that this loss is very small. Two calomel ointments differing in facility of penetration

TABLE 2

The percentage distribution in tissues and excreta of the total amount of mercury entering the body of the rat via the skin

EXPOSURE	RAT NO.	TOTAL AMOUNT OF MERCURY*	PER CENT OF THE TOTAL AMOUNT OF MERCURY				
			Carcass	Kidneys	Liver	Blood	Excreta
			Ointment A				
hours		*micrograms*					
24	399	41	49	21	10		19
	400	38	50	26	9.3		13
	401	44	50	32	10		8.6
Averages.			50	26	9.7		13.5
48	402	89	31	37	7.1		25
	403	117	28	44	5.9		22
	404	124	35	36	6.5		23
Averages.			31	39	6.4		23
			Ointment B				
24	437	145	44	33	6.9	3.1	12
	438	121	40	34	5.4	4.9	17
	439	68	46	22	5.6	2.5	25
Averages			43	30	6.0	3.5	18
48	440	105	33	35	5.9	2.4	23
	441	265	38	34	3.5	2.3	23
	442	169	28	51	4.3	1.8	15
Averages			33	40	4.6	2.2	20

* Consisting of the sum of carcass, kidneys, liver, blood, and excreta.

were used, and the exposures made for 24 and 48 hours. The results are shown in table 2. It is evident from the column headed "Total Amount" that ointment B supported higher penetration of mercury. The per cent distribution of mercury in the tissues, however, is essentially the same for both ointments. On the other hand, when the exposure period is extended 24 hours beyond the usual, a well-defined change in distribution occurs. The trend to lower values can be seen in carcass, liver and blood, the loss of mercury in these tissues being roughly

equalled by the gain in kidney and excreta. This would indicate that the store of mercury in the kidney is not being rapidly dissipated, but rather continues to build up in spite of some increase in excretion. Finally, it should be emphasized that the per cent of the total in the kidneys is nearly twice that in the excreta. Aside from this obvious advantage, the technical reasons for using kidney tissue instead of excreta are even greater than are apparent from the data. It should be remembered that the average pair of rat kidneys weigh only 2 grams and are easily removed without danger of contamination, while a 24-hour sample of excreta weighs about 50 times as much and may be very subject to contaminaation errors.

VOLATILITY. Although there was reasonable assurance that the type of shield described effectively prevented oral contamination, it was not certain to what extent mercury volatilizing from the ointment in contact with the skin would be inhaled by the experimental animal during the 24-hour exposure period. Tests had shown that the celluloid itself was impervious to mercury, but it was not expected that the celluloid-to-skin seals would be entirely gas-tight. Therefore, it was conceivable that mercury-vapors arising under the shield could be inhaled. To test volatility under the conditions of the cutaneous exposure, the rat was prepared as usual but, instead of applying the ointment to the skin, it was applied to the outer surface of a celluloid shield fastened to the skin. This was then covered in the usual manner with the complete shield as described above. For comparison, control animals were wrapped in shields, but without mercury, and in addition a third group of animals was exposed to mercury ointment on the skin. Two different mercury ointments were used, calomel and metallic (Blue ointment), each containing 25 per cent mercury. The latter was chosen because of indications that this preparation would give the highest mercury vapor tension. The results are shown in table 3. It can be seen that the tissues from the shield-inuncted animals contain significantly greater amounts of mercury than the controls, and this is true without exception in the kidneys. The interpretation of these results is, therefore, that some mercury is volatilized from the site of inunction and presumably inhaled, although differences between the volatility of the two mercury ointments is not revealed by these tests. Referring to the mercury stored in the kidneys following cutaneous inunction, it can be calculated that of this amount, slightly less than 4 per cent has come from inhalation. If we observe, however, that there is considerable variability between animals in mercury storage, it may be concluded that the volatility error is of minor importance.

Certain factors may be expected to influence not only the amount of mercury which penetrates the skin, but also the amount which is taken up by the kidney Some of the factors to be considered, such as, for example, sex, litter mate and species, may directly influence mercury storage in the kidney since they frequently reflect organic differences. In any event it is desirable, particularly in a biological assay, to recognize the effects of as many variants as possible. With this knowledge, conditions can then be set which will produce the greatest constancy of result.

Effect of covering and site of inunction area. According to Rothman (14)

covering the skin results in interference with the normal escape of moisture. Moisture thus accumulated may produce softening and maceration of the stratum corneum, a condition which is favorable to the retention of substances in close contact with the skin, and possibly also to penetration of the skin. While the factor of covering the skin can hardly be considered as a variable in these experiments, since the skin was usually covered in order to avoid contamination, it seemed desirable to test the effect of leaving the inuncted skin uncovered during the 24-hour exposure period. The experiments here reported were done on rabbits, since the maintenance of these animals in a restraining holder did not make an occlusive shield obligatory, as in the case of the rats.

As stated in the procedure, all inunctions were made on skin of the back and

TABLE 3

The volatility of mercury ointments under the condition of cutaneous exposure as measured by the mercury content of liver and kidney of the rat

(Micrograms per gram of wet tissue)

SKIN INUNCTION		SHIELD INUNCTION		CONTROL	
Kidney	Liver	Kidney	Liver	Kidney	Liver
Calomel					
14.2	0.42	0.63	0.05	0.10	0
14.0	0.64	0.44	0.04	0.09	0.01
20.0	1.07	0.87	0.19	0.08	0.02
13.1	1.16	0.57	0.17	0	0
Avg.....15.3	0.82	0.63	0.11	0.07	0.01
Metallic mercury					
20.1	0.58	0.72	0.02	0.10	0
10.1	0.65	0.69	0.15	0.11	0.04
9.8	0.64	0.57	0.10	0.37	0.02
7.7	0.43	0.53	0.02	0.08	0
Avg.....11.9	0.58	0.63	0.07	0.16	0.02

flanks. It appeared, however, that the belly skin of the rabbit was thinner and possibly less resistant to penetration. Exposure to back and belly skin was, therefore, compared in the same series of rabbits in which the effect of covering was examined. The results are shown in table 4. Six different ointments were used, and two rabbits for each treatment. The results are expressed as averages of the mercury concentrations in the kidneys of the two animals exposed to each treatment. It is clear that covering the inuncted skin area produced a marked increase in the amount of cutaneous penetration of mercury, which varies somewhat with the type of ointment. On the other hand, changing the site of inunction from back to belly produces no significant difference in penetration of mercury.

Effect of species difference and size of inunction area. As noted above, the size of the inunction area was fixed as a constant for both rats and rabbits. Since it was noted, however, that some of the ointment occasionally found its way across the boundaries of the inunction area, it became necessary to investigate to what extent such variation in effective penetrating area might be responsible for occasional large and unexplained variation in mercury storage in the kidney. This study was conducted simultaneously on groups of rats and rabbits, and the effect of halving the standard inunction areas was observed on the storage of mercury in kidneys and livers of each species. Two ointments of widely different penetrating abilities were used. The average values for 6 animals on each treatment are given in table 5. Comparing first the effect of difference in species, it can be seen that within the sensitivity of this assay procedure, no difference between the rat and rabbit can be demonstrated when approximately equal percentages of the total skin area are exposed. When the area of exposure was halved, the respective storage response of the kidneys was not halved, but reduced by approximately one-third. This would indicate that some latitude in area variation can be tolerated before measurable effects on absorption are detectable by this method.

TABLE 4

The effect of covering the inuncted skin area and changing the site of inunction on the cutaneous penetration of mercury in the rabbit

Storage of mercury in the kidney is used as a measure of penetration (Micrograms mercury per gram wet kidney tissue)

CALOMEL OINTMENT	UNCOVERED* SKIN OF BACK	COVERED* SKIN OF BACK	COVERED* SKIN OF BELLY
1	7	26	35
2	13	45	38
3	8	17	27
4	9	20	18
5	4	6	7
6	5	22	26
Avg.	8	23	25

* Average of two animals on each treatment.

Effect of removal of excess ointment. In these studies it has been the practice not to remove the excess ointment after inunction, but to allow it to remain in contact with the skin for 24 hours. Clinically, the use of the "clean" inunction, i.e. removal of excess with benzene has found some favor, but Cole and coworkers (15) report that the penetration of mercury is somewhat less. In our experiments, removal of excess ointment was effected by simply wiping the skin clean with paper tissue. The difference between "clean" and "ordinary" inunction was quite marked. Thus, in six rats in which the ointment was removed immediately after inunction, the storage of mercury in the kidneys averaged 17

micrograms per gram, while in a corresponding group in which the ointment remained on the skin for 24 hours, storage averaged 27 micrograms per gram. The significantly lower penetration of mercury following removal of excess ointment is not unexpected, and in keeping with the idea that penetration of mercury from a depot in and on the skin depends in part on the size of that depot.

Effect of washing the skin before application of ointment. Ordinarily the openings of the skin, such as the hair shafts and sebaceous ducts, which are regarded as the avenues of entrance for mercury, may be partially occluded by the presence therein of cell detritus and oily secretions. Cleansing of the skin with soap and

TABLE 5

The effect of size of inunction area and animal species on the cutaneous penetration of mercury as measured by its concentration in liver and kidney

OINTMENT	BODY		INUNCTION		MERCURY CONCENTRATION	
	Weight	Surface	Area	Ratio—AREA/BODY SURFACE	Liver	Kidney
Rabbit*						
	grams	*(cm.)²*	*(cm.)²*		*micrograms per gram wet weight*	*micrograms per gram wet weight*
C	2803	2147	155	0.0722	0.31	4.5
	2742	2116	77.5	0.0366	0.24	2.3
Rat*						
C	209	320	29	0.0906	0.30	5.3
	205	316	14.5	0.0459	0.20	3.6
Rabbit*						
D	2482	1980	155	0.0783	1.24	25
	2607	2046	77.5	0.0379	0.86	18
Rat*						
D	209	320	29	0.0906	1.09	29
	212	323	14.5	0.0449	0.78	23

* The results are averages of 6 animals on each treatment.

water would be expected to remove some of this "plugging" material, without the more rigorous effects which follow the application of lipoid solvents such as alcohol or ether. However, a comparative study of washed and unwashed skins in two groups of rats showed definitely that treatment of the skin with soap and water prior to inunction had no effect on cutaneous penetration of mercury as measured by storage of the metal in the kidney, even though the cleaning operation was seen to remove a visible yellowish scale of dander-like material.

Effect of weight, sex, and litter. The studies were generally conducted on groups of animals homogeneous with respect to age, weight and sex, but heterogeneous

with respect to litter. As examples of 3 possible variants, litter, sex and weight were studied. It may be presumed that these variants could operate to produce differences between skins, as characterized by such factors as texture, thickness, subcutaneous fat, number of hair follicles, etc., or differences between organs such as the ability of the kidney to excrete or store mercury. The 24 animals used in this study originated from 6 different litters and were 85 days of age. The results are given in table 6. An analysis of variance shows that the cutaneous penetration of mercury as judged by the level in the kidneys is not significantly affected by weight, sex or litter.

TABLE 6

The effect of weight, sex and litter on the cutaneous penetration of mercury and its storage in the kidney of the rat

MALES			FEMALES		
Body weight	Litter no	Micrograms mercury per gram wet kidney	Body weight	Litter no.	Micrograms mercury per gram wet kidney
grams			*gram*		
185	5	13	145	5	5.0
210	6	5.6	150	5	18
230	4	9.9	165	4	5.9
235	4	11	170	2	8.0
240	6	6.6	175	2	5.2
245	6	12	180	4	5.0
250	2	8.7	195	6	2.0
265	2	6.5	195	6	2.8
285	1	3.2	200	3	23
290	1	11	210	1	6.8
300	3	10	210	1	7.0
310	3	8.4	255	3	12
Avg..		8.8			8.4

SUMMARY

1. A method for determining cutaneous penetration of mercury is described which depends on the measurement of the quantity of mercury which is stored in the kidney during 24 hours of exposure.

2. Using the storage of mercury in the kidney as a measure of how much has passed through the skin, a number of factors have been examined for their influence on the cutaneous penetration of mercury.

(a) Covering the inunction site increases penetration nearly four-fold.

(b) The location of the inunction site appears to have no effect on penetration.

(c) Penetration of mercury through approximately equal percentage areas of total body surface is the same for the rat and the rabbit.

(d) Removal of excess ointment from the skin (clean inunction) reduces the amount of penetration.

(e) Washing the skin with soap and water before inunction has little or no effect on penetration.

(f) Halving the exposure area reduces penetration by approximately one-third.

(g) Sex and litter of the assay animals were found to have little or no measurable influence on penetration.

3. Due to the volatility of mercury compounds, a small but detectable amount of mercury is inhaled under the conditions of cutaneous exposure.

REFERENCES

(1) Sussmann, P. O.: Arch. f. Hyg., 90: 175, 1922.

(2) Cole, H. N., Schreiber, N., and Sollmann, T.: Arch. Dermatol. and Syphilol., 21: 373, 1930.

(3) Cole, H. N., DeWolf, H. F., Schreiber, N., Sollmann, T., and Cleve, J. V.: Arch. Dermatol. and Syphilol., 27: 1, 1933.

(4) Sollmann, T., Cole, H. N., and Schreiber, N. E.: Arch. Dermatol. and Syphilol., 32: 242, 1935.

(5) Wile, U. J., and Elliott, J. A.: J. Am. Med. Assoc., 68: 1024, 1917.

(6) Moncorps, C.: Arch. f. exper. Path. u. Pharmakol., 155: 51, 1930.

(7) Buchtala, H.: Ztschr. f. Physiol. Chem., 83: 249, 1913.

(8) Lomholt, S.: Arch. f. Dermatol. u. Syphilis, 126: 1, 1918.

(9) Stock, A., and Cucuel, F.: Angew Chemie, 47: 801, 1934.

(10) Kluge, H., Tschubel, H., and Zitek, A.: Ztschr. f. Untersuch, d. Lebensmit., 76: 321, 1938.

(11) Laug, E. P., and Nelson, K. W.: J. Assoc. Off. Agric. Chem., 25: 399, 1942.

(12) Lusk, G.: The Science of Nutrition, p. 122–3 4th Ed. 1928. W. B. Saunders Co., Phila.

(13) Laug, E. P.: J. Lab. and Clin. Med., 29: 308, 1944.

(14) Rothman, S.: Arch. Dermatol. u. Syphilis, 131: 549, 1921.

(15) Cole, H. N., Gammel, J. A., Rauschkolb, J. E., Schreiber, N., and Sollmann, T.: Arch. Dermatol. and Syphilol., 17: 625, 1928.

A STUDY OF CERTAIN FACTORS GOVERNING THE PENETRATION OF MERCURY THROUGH THE SKIN OF THE RAT AND THE RABBIT[1]

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Received for publication September 16, 1946

In connection with the development of methods for cutaneous prophylaxis against venereal disease, it was necessary to examine a large number of mercury ointments, chiefly those containing calomel. The criterion of efficiency was the ease with which cutaneously applied mercury penetrated the skin. The following studies are an attempt to evaluate some of the factors which influence penetration of mercury.

METHOD. The storage of mercury in the kidney of the rat and the rabbit was used as a means of assaying the amount which had penetrated the skin. This procedure has been fully described in the preceding paper (1). Mercury determinations of tissues were carried out by a method developed in our laboratories (2).

The ointments presented for evaluation contained 30 per cent calomel which was suspended in vehicles representative of three generally recognized classes, namely, fat, water-in-oil, and oil-in-water. It soon became apparent, however, after a number of assays had been made, that, while it was easily possible to demonstrate marked differences in penetration of mercury from different types of vehicles, it was not easy to explain these differences. This was so because the ordinary multicomponent ointment presented such a formidable array of possible variables affecting penetration of mercury, that the task of unravelling these would become hopeless. In an attempt to simplify the problem, two methods of approach were used. (a) Construction of single or double component vehicles. By this means it was possible to determine not only the influence of the vehicle, but that of the compound of mercury on the penetration of mercury through the skin under conditions subject to the least number of variables. (b) The varying of one important constituent or its properties in a complete ointment. The following were studied: (1) State of physical subdivision of calomel; (2) Concentration of mercury; (3) The addition of wetting agents; (4) The addition of sulfathiazole.

RESULTS. Some of the common constituents ordinarily combined in multicomponent vehicles were examined separately to determine their effect on the penetration of mercury. In table 1 are shown the results of the inunction of calomel suspended in 30 per cent concentration in these "single component" vehicles. Two separate assays were made on a group of male and female rats with a time interval of approximately one month intervening. It is apparent from the results that the two series show close agreement. Referring to the average kidney values for the storage of mercury in both series, it can be seen

[1] A portion of the funds used in this investigation was supplied by a transfer, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Division of Pharmacology of the Food and Drug Administration.

that the smallest skin penetration occurs from a suspension of calomel in water, the largest from a suspension of calomel in oleic acid. Petrolatum, mineral oil, anhydrous lanolin and hydrous lanolin all support the same penetration and do not differ significantly among themselves in kidney level of mercury. The water suspension of calomel, while showing a tendency toward lower penetration than, for example, petrolatum, does not actually differ significantly in kidney level

TABLE 1

The cutaneous penetration of mercury when applied as a 30 per cent suspension of calomel in a number of "single substance" vehicles

The measure of penetration is the storage of mercury in the kidneys of individual rats

(Micrograms mercury per gram wet kidney)

CONTROL	WATER	PETROLATUM	MINERAL OIL	ANHYDROUS LANOLIN	HYDROUS LANOLIN	CORN OIL	LARD	PROPYLENE GLYCOL	OLEIC ACID
Series A, females									
	3.5					13		20	34
	5.7	7.6		7.9	5.5	18	13	19	30
0.10	3.9	5.1	4.6	7.4	8.0	16	14	19	24
0.09	3.7	—	6.6	4.7	4.8	11	7.9	18	23
0.08	3.7	8.8	6.6	3.3	5.0	13	7.0	17	24
0	6.1	2.7	4.7	6.7	6.4	8.1	11	18	21
Avg.. . 0.07	4.4	6.0	5.6	6.0	5.9	13	11	19	26
Series B males									
	2.1	8.8	7.9	4.8			15	15	
	7.6	3.7	6.7	7.7		12	8.2	13	
0.10	5.6	8.2	7.7	5.9		11	12	18	
0.11	5.1	2.8	3.6	5.2		11	12	17	
0.37	1.4	3.5	2.7	3.1		6.0	9.0	21	
0.08	2.8	6.4	2.6	7.1		16	4.8	9.1	
Avg.....0.17	4.1	5.6	5.2	5.6		11	10	16	
Grand avg... 0.12	4.3	5.8	5.4	5.8		12	10	17	

($p > 0.05$). At the higher penetrations the differences between the kidney levels following oleic acid and propylene glycol are highly significant, ($p = 0.001$), and similarly between propylene glycol and lard. Lard and corn oil, the intermediate penetration, while not differing significantly between themselves, show kidney values that are significantly higher than, for example, petrolatum ($p = 0.001$). One of the most striking points brought out by this experiment is that petrolatum, mineral oil and lanolin, which have found such extensive use as con-

stituents for ointment bases, support a penetration of mercury which is not markedly greater than if the calomel is applied merely as a simple paste with water.

From the "single component" vehicles, 4 two-component vehicles were prepared as follows: Lard + anhydrous lanolin, propylene glycol + petrolatum, mineral oil + anhydrous lanolin, corn oil + petrolatum. The results are computed as averages in table 2, with the values for the separate components added from table 1 in order to facilitate comparison. It would appear that when a substance which gives good penetration of mercury is mixed with one which gives poor penetration, the properties of the combination resemble the former rather than the latter, as shown with vehicles A, C and D. On the other hand, vehicle

TABLE 2

The cutaneous penetration of mercury when applied as a 30 per cent suspension of calomel in "double" component vehicles

The measure of penetration is the storage of mercury in the kidneys of the rat

	"DOUBLE" COMPONENT VEHICLE	KIDNEY*	"SINGLE" COMPONENT VEHICLE	KIDNEY
		micrograms mercury per gram wet weight		*micrograms mercury per gram wet weight*
A	50% Lard 50% Anhydrous lanolin	11	Lard	10
B	50% Mineral oil 50% Anhydrous lanolin	5.9	Anhydrous lanolin	5.8
			Mineral oil	5.4
C	50% Corn oil 50% Petrolatum	10	Corn oil	12
D	50% Propylene glycol 50% Petrolatum	14	Petrolatum	5.8
			Propylene glycol	17

* Averages of 6 animals.

B gives penetration of mercury which is indistinguishable from that of either component.

Four different mercurials, ammoniated mercury, metallic mercury, yellow oxide of mercury and calomel were suspended respectively in each of two double component bases consisting of equal parts of lard and lanolin and equal parts of petrolatum and lanolin, the former mixture being the same as vehicle A in table 2. These vehicles were selected for study because they frequently form the main base for a number of commonly used ointments. The concentration of mercury was 25 per cent in all cases. In table 3 is shown the marked superiority of the lard-component vehicle over the petrolatum-component vehicle. The increase in penetration of mercury from calomel, metallic and yellow oxide is roughly two-fold, but in the case of ammoniated mercury the increase is nearly four-fold. With regard to the differences between compounds of mercury, inspection shows

that yellow oxide gives the highest and calomel the lowest penetration of mercury in both vehicles. This is supported by statistical analyses of the results, but with two exceptions: In vehicle A the difference between ammoniated mercury and yellow oxide is not significant, $p > 0.05$, and in vehicle E the difference between calomel and ammoniated mercury is not significant, $p > 0.05$. It is to be noted especially that while the difference between calomel and ammoniated mercury is not significant in vehicle E, it is highly significant in vehicle A, $p < 0.002$. Presumably this marked effect of the vehicle may be attributed to some interaction with the compound as has been suggested by Moncorps (3) who observed that the stability of this compound was somewhat uncertain. It is, therefore, not unlikely that the reactivity of a compound with its vehicle may be one of the causes for differences between compounds. This is indicated in table 4 where mercuric oleate in oleic acid and calomel in oleic acid have been compared. It can be seen that there is no distinction between the two compounds, and it would appear therefore, that the vehicle rather than the compound may be the more important factor in determining cutaneous penetration.

TABLE 3

Comparison between two types of vehicles consisting of lard and petrolatum with lanolin as common constituent on the pentration of four different mercurials

The concentration of mercury in all ointments is 25 per cent. The measure of penetration is the storage of mercury in the kidneys of individual rats

(Micrograms mercury per gram wet kidney)

VEHICLE	CALOMEL	AMMONIATED MERCURY	METALLIC MERCURY	YELLOW OXIDE MERCURY
E 50% Petrolatum	4.9	8.1	9.5	15
50% Lanolin	2.8	5.9	9.8	9.1
	3.7	4.2	8.8	13
	5.8	4.2	6.6	9.6
	5.7	5.3	8.6	8.5
	2.5	—	4.4	11
Averages..	4.2	5.5	7.9	11
A 50% Lard	9.9	21	18	16
50% Lanolin	13	18	17	22
	4.6	24	12	34
	8.7	12	12	13
	9.1	16	12	33
	7.5	20	15	18
Averages.............	8.8	19	14	23

Effect of wetting agents. Because of the well known properties of wetting agents, it seemed an attractive possibility that their addition to the ointment base might modify the percutaneous penetration of mercury. Three different types of vehicles, with and without 2 per cent Triton NE (an aryl alkyl polyether

alcohol) were studied. The calomel concentration of the finished ointment was 30 per cent. The results are shown in table 5. Vehicle A_1, except for the addition of antioxidant and slight amount of beeswax as stiffening agent, was the same as vehicle A shown in tables 2 and 3. This vehicle was selected because it represented a pure animal fat base, presumably reactive with the mercurial under certain conditions. Vehicle F was a pure mineral base, chosen because it is generally considered unreactive. Vehicle G was an oil-in-water emulsion type, chosen because its properties might be expected to be most modified by the presence of the wetting agent.

It is indicated that addition of Triton NE to vehicles A_1 and G markedly increases skin permeability to mercury but has no effect on vehicle F. The effect in G may perhaps best be explained by the fact that this vehicle contains a large

TABLE 4

Comparison between the cutaneous penetration of mercury from mercuric oleate and from calomel both suspended in oleic acid

The concentration of mercury in the two ointments is 25 per cent. The measure of penetration is the storage of mercury in the kidneys of individual rats

(Micrograms mercury per gram wet kidney)

	MERCURIC OLEATE IN OLEIC ACID	CALOMEL IN OLEIC ACID
	29	34
	53	30
	25	24
	31	23
	32	24
	33	21
	22	29
	25	49
	24	44
	40	40
Avg.	31	32

proportion of water and water soluble substances whose relationship to the fatty constituents is altered by the presence of the wetting agent.

Using vehicle A_1, a second series of determinations were made with the addition of three different wetting agents, namely, Triton NE, Aerosol OT (a sulfonated ester of di octyl succinate), and Duponal C (sodium lauryl sulfate). The results are shown in table 6. It can be seen that discrimination between wetting agents is lacking and further that no significant difference between 2 per cent and 0.5 per cent Triton NE is discernible. The latter observation may, however, merely indicate that the optimum concentration of the wetting agent in the vehicle is 0.5 per cent or less.

Effect of sulfathiazole. Since it was proposed to include 15 per cent sulfathiazole with the calomel ointments, it seemed desirable to investigate not only

TABLE 5

Cutaneous penetration of mercury as affected by the addition of Triton NE to three different types of vehicles

The measure of penetration is the storage of mercury in the kidneys of individual rats

(Micrograms mercury per gram of wet kidney)

	OINTMENT A_1		OINTMENT F		OINTMENT G	
	Without wetting agent	With 2% wetting agent	Without wetting agent	With 2% wetting agent	Without wetting agent	With 2% wetting agent
	7.4	7.6	6.3	11	4.1	17
	11	17	6.5	7.6	7.6	9.4
	9.3	16	6.0	5.4	5.5	6.2
	6.1	19	4.7	4.8	7.2	8.4
	10	10	9.6	9.8	5.5	13
	13	12	1.9	7.6	5.2	15
	5.9	13	4.2	5.5	12	22
	10	11	6.4	4.3		
Avg....	9.1	13	5.7	7.0	6.7	13

Difference between groups

significant $p = 0.03$	not significant $p > 0.05$	significant $p = 0.02$
Composition of vehicle A_1	**Composition of vehicle F**	**Composition of vehicle G**
Anhydrous lanolin 50%	White petrolatum. 100%	Propylene glycol . 65.1%
d-isoascorbic acid palmitate 0.1%		Cetyl alcohol . 3.7%
Lard. . . 47.8%		Stearic acid . .. 4.0%
Beeswax.. ... 2.1%		Glycerol monostearate . 17.0%
		KOH . 0.18%
		Borax 0.02%
		Water 10.0%

TABLE 6

Cutaneous penetration of mercury as affected by the addition of different wetting agents to vehicle A_1

The measure of penetration is the storage of mercury in the kidneys of individual rats

(Micrograms mercury per gram wet kidney)

	0.5% TRITON NE	2.0% TRITON NE	2.0% AEROSOL OT	2.0% DUPONOL C
	22	9.1	19	8.5
	12	14	12	19
	20	23	23	11
	17	26	9.5	18
Avg.	18	18	16	14

whether change in the physical characteristics of the ointment would have any effect on the penetration of mercury but also whether the presence of the sulfur in sulfathiazole might induce the formation of mercury compounds having modified penetrating properties. Seven pairs of different ointments, with and without 15 per cent sulfathiazole, were studied. The vehicles of these ointments covered the entire range of types presented to us for assay, namely, animal fat, mineral oil or petrolatum base, water-in-oil, or oil-in-water emulsions. The addition of sulfathiazole was affected without reducing the 30 per cent calomel concentration. Generally speaking, raising the solid content of the ointment by an additional 15 per cent had a tendency to produce drier and less unctuous ointments. Rabbits were used, and in addition to kidney tissue a block of skin 2.5 (cm.)² from the area exposed was removed with a punch and analyzed for mercury. Before sampling,

TABLE 7

Effect of addition of 15 per cent sulfathiazole to 7 different calomel ointments on the cutaneous penetration and concentration of mercury

The measure of penetration is the storage of mercury in kidney tissue of the rabbit

VEHICLE TYPE	WITHOUT SULFATHIAZOLE*		WITH SULFATHIAZOLE*	
	Kidney (micrograms mercury per gram wet weight)	Skin (micrograms mercury per (cm)² wet skin)	Kidney (micrograms mercury per gram wet weight)	Skin (micrograms mercury per (cm)² wet skin)
Water-in-oil	6.6	13	3 4	53
Water-in-oil	9 2	16	6.4	22
Oil-in-water	5 8	4 4	3 6	6 8
Oil-in-water	3 4	4 0	3 7	6.8
Oil-in-water	10	1 7	6 8	17
Petrolatum base	5 8	0 68	4 5	31
Animal fat	5 3	3 0	4.6	12
Average	6 6	6 1	4 7	21

* One animal only was used for each treatment

all excess ointment was first removed by scraping, following which the skin was thoroughly scrubbed with soap and water. Table 7 shows the results. It is indicated that the presence of sulfathiazole reduces the penetration of mercury but it is not possible from this experiment to decide clearly whether sulfathiazole modified the passage of mercury through the skin or its disposition once it had gained entrance. That the former explanation is the more probable one is supported by the finding that the skin shows markedly greater amounts of mercury after calomel-sulfathiazole inunction than after calomel. This may indicate that the sulfathiazole has immobilized some of the mercury within the skin structures in either a chemical or a physical manner.

To decide to what extent the mechanical "stiffening" of an ointment due to sulfathiazole may influence the penetration of mercury, an attempt was made to control the mechanical factor by the construction of an ointment containing 15

per cent talc as an inert filler. The particular vehicle selected was of the type which would be expected to show the most marked "stiffening" effects from the addition of a filler. The results are shown in table 8. While the difference between calomel with and without sulfathiazole is distinctly significant, $p = 0.007$, this is not so for the difference between calomel with and without talc. Actually talc gives a significantly higher penetration than sulfathiazole ($p = 0.007$). One may be led to conclude from these limited data that the mechanical factor from sulfathiazole may be of relatively minor importance on its influence on the percutaneous penetration of mercury.

State of subdivision of calomel. Distinction was made between two different calomel powders on the basis of particle size: (a) Ordinary fine milled: Range of

TABLE 8

The effect of sulfathiazole and talc on the cutaneous penetration of mercury through the skin of individual rats

The measure of penetration in the storage of mercury in liver and kidney tissue (Micrograms mercury per gram of wet tissue)

TALC PLUS CALOMEL		CALOMEL		SULFATHIAZOLE PLUS CALOMEL	
Liver	Kidney	Liver	Kidney	Liver	Kidney
0.57	13			0.53	12
0.61	14	0.79	18	0 57	12
0.57	17	0 37	16	0.43	12
0.56	13	0.74	13	0.50	8.5
0.63	19	0.67	24	0.39	6.0
0.44	17	0.74	22	0 46	13
Avg... 0.56	15	0.66	19	0.48	11

Composition of vehicle:	%
Propylene glycol	26
Starch glycerite	34
Stearic acid	4
Glycerol monostearate	2
Spermaceti	2
Water	32

size 10 to 100 micra; (b) Micronized: Range of size 1 to 10 micra. Four different types of vehicles were used, oil-in-water, water-in-oil, 100 per cent petrolatum, 100 per cent propylene glycol. In selecting the vehicles, cognizance was taken of the possibility that the effect (if any) of particle size on penetration might be related to aqueous solubility of the finely divided calomel. Hence, two of the vehicles contained water and propylene glycol. Table 9 shows the results. It is clear that better penetration is obtained with micronized calomel, but the effect is not favored by any particular vehicle. Possibly the better penetration of mercury from the ointments containing micronized calomel may result from the greater facility with which these small particles can be forced into the skin appendages by inunction.

Concentration of mercury. Since the application of mercurial ointments was always designed to leave an excess on the skin, the question arose whether reduc-

TABLE 9

Effect of particle size of calomel on the cutaneous penetration of mercury

The measure of penetration is the storage of mercury in kidney tissue of the rabbit and the rat

(Micrograms mercury per gram wet kidney)

TYPE OF VEHICLE	TYPE OF CALOMEL	
	Ordinary fine milled (particle size. 10–100 micra)	Micronized (particle size: 1–10 micra)
Oil-in-water*	18	35
Water-in-oil*	11	32
100% petrolatum*	16	26
100% propylene glycol†	15	28

* Average of two rabbits on each treatment.

† Average of six rats on each treatment.

TABLE 10

Effect of mercury concentration in the vehicle on the cutaneous penetration of mercury

The measure of penetration is the storage of mercury in the kidney tissue of individual rats

(Micrograms mercury per gram of wet kidney)

	CALOMEL		METALLIC MERCURY			AMMONIATED MERCURY		YELLOW OXIDE MERCURY	
	Per cent metallic mercury								
	25	8.3	50	25	10	25	4	25	0.93
	6.3	10							
	5.4	7.5	15	12	13	14	18	14	9.1
	7.3	10	16	16	18	13	14	12	8.4
	8.6	3.1	17	10	6.5	9.4	7.7	12	9.7
	9 4	6.7	8.0	14	9.7	11	5.8	13	6.0
	7.0	4.1	6.1		8.4		8.8		3.3
	11	6.4	9.8		7.3		11		5.4
Avg.	7.9	6.8	12	13	10.5	12	11	13	7.0
	Vehicle		Vehicle			Vehicle		Vehicle	
	Petrolatum . 100%		Oleate of mercury . 4%; Lanolin . 60%; White wax 10%; White petrolatum 26%			Anhydrous lanolin.. 5.3%; White petrolatum.. 94.7%		Anhydrous lanolin.. 1.0%; Yellow petrolatum.. 99.0%	

tion in concentration of the mercurial might be effected without seriously reducing its cutaneous penetration. The possibilities of conserving mercury by this means

were obvious. Four different mercurials were examined, of which three, the metallic 50 per cent and 10 per cent, ammoniated 4 per cent, and yellow oxide 0.93 per cent are official U.S.P. preparations. Adjustments were made to give a large range of concentration of mercury. The results are given in table 10. With the exception of the yellow oxide, where a 25-fold increase in mercury produced a significant rise in cutaneous penetration, it can be seen that the response of cutaneous penetration to changes in concentration of mercury in the other ointments is relatively insensitive. It should be remarked, however, that this result might not be so unequivocal with other techniques of application where contact with mercury is repeated daily or prolonged beyond the 24-hour exposure period used in these assays. In some experiments with rabbits in which 10 and 25 per cent calomel ointments were compared by a modified 4-day exposure technique, significantly more mercury was found in the kidneys of the animals treated with the higher concentration. Similarly, when the carefully cleaned skin from areas exposed to 10 and 25 per cent calomel were analyzed and compared, it was found that the former averaged 24, and the latter 51 micrograms of mercury per 2.55 $(cm.)^2$ of skin.

Effect of aging. The results of Menschel (6) have indicated that interaction between mercury and ointment base takes place with the formation of mercuric oleate. According to our results, mercuric oleate gives by far the best penetration. Table 11 illustrates quite effectively that mercury may react with lard and presumably forms mercuric oleate with the oleic acid. It can be seen that after approximately 16 months of contact between calomel and lard, significantly greater penetration of mercury occurred than from a freshly prepared ointment. Because of the possibility that other factors might modify the assay and falsify the interpretation, a calomel in petrolatum assay was run simultaneously as control. This shows that none of the conditions of the assay had changed and that it could be permissible to interpret the results with the lard ointment as due to some type of interaction.

DISCUSSION. From the data presented it would appear that the vehicle is the most important factor in the cutaneous penetration of mercury. One of the time honored vehicles, petrolatum, has been shown to support only relatively small penetration of mercury, while lard, in contrast, proved to be an excellent vehicle. It is possible that the difference between lard and petrolatum may lie in the oleic acid content of the former. There is reason to believe that by interaction with the vehicle, some compounds of mercury may be converted to the oleate. Mercuric oleate itself stands far above all the other compounds of mercury studied in its penetrating properties.

While the device of studying very simple vehicles has pointed the way toward the construction of more complicated vehicles which may be presumed to be effective in supporting good penetration of mercury, one must not lose sight of the possibility that interaction between vehicles, resulting in chemical and mechanical changes, may so outweigh the properties of the individual substance that its effects become in fact unrecognizable. Generally speaking, it would appear that the type of vehicle, whether fat, water-in-oil or oil-in-water emulsion, is less im-

portant to penetration of mercury than the constituents which it contains. Thus, petrolatum, mineral oil or lanolin cannot be regarded as favoring the penetration of mercury in any vehicle, while oleic acid or propylene glycol, provided they are not too strongly diluted, may be expected to enhance penetration.

In contrast to the results of Cole et al. (4), which unequivocally favor a high concentration of mercury in the ointment in order to accomplish high penetration, our assay techniques do not bear this out. This is best explained by the fact that the exposure period in the rat assay method lasts but 24 hours while the

TABLE 11

Aging of a lard-calomel ointment and its effect on the cutaneous penetration of mercury

The measure of penetration is the storage of mercury in the liver and kidney tissue of the rat (Micrograms mercury per gram wet tissue)

30% CALOMEL, 70% BENZOINATED LARD. PREPARED 11-8-43, ASSAYED 11-11-43		30% CALOMEL, 70% PETROLATUM. PREPARED 11-8-43, ASSAYED 11-11-43	
Kidney	Liver	Kidney	Liver
13	0.68	8.8	0.65
14	0.70	3.7	0.38
7.9	0.35	8.2	0.41
7.0	0.36	2.8	0.36
11	0.45	3.5	0.22
		6.4	0.30
Avg.....11	0.51	5.6	0.39
SAME OINTMENT. REASSAYED 4-26-45		FRESHLY PREPARED AND ASSAYED 4-26-45	
15	0.99	10	0.52
24	0.72	6.5	0.41
19	0.82	4.4	0.40
13	0.51	6.4	0.34
20	0.71	2.4	0.21
14	0.95	6.6	0.40
11	0.56	2.2	0.34
Avg......17	0.75	5.5	0.37

human assay method extends over weeks during which time there is a better chance for the larger reservoirs of mercury to make themselves evident in urinary and fecal excretion. Conversely, however, the prolonged exposure experiments of Cole et al. fail to discriminate between particle size and vehicle type, both of which we have shown to be so effective in determining penetration.

The studies on the effects of wetting agents on penetration are at best not very striking compared with those obtained by Duemling (5) with other penetrating substances. The relationship between the vehicle and the wetting agent must obviously be subjected to additional intensive study.

SUMMARY

1. The vehicle appears to have the greatest influence on the penetration of mercury. Of the single component vehicles, oleic acid and propylene glycol support the greatest penetration of mercury. With the exception of water, the poorest vehicles are the ones which have been most frequently incorporated in ointments, namely, petrolatum, mineral oil and lanolin.

2. Next to the vehicle, the compound of mercury appears to have the greatest influence on the cutaneous penetration of mercury.

3. Cutaneous penetration is least affected by the concentration of mercury in the ointment. In the case of the yellow oxide of mercury, it requires a 25-fold increase in concentration in the ointment to cause a doubling of penetration.

4. Reduction in particle size of calomel increases the penetration of mercury.

5. Addition of 15 per cent sulfathiazole to a calomel ointment reduces cutaneous penetration of mercury by approximately one-third.

6. The type of vehicle appears to determine the effectiveness of the wetting agent in increasing the penetration of mercury through the skin.

7. Evidence is presented to show that interaction between the mercury compound and the vehicle may materially modify penetration through the skin.

The authors wish to thank Mr. O. L. Evenson, of the Cosmetic Division, for the preparation of some of the ointments.

REFERENCES

(1) Laug, E. P., Vos, E. A., Umberger, E. J., and Kunze, F.: This Journal (In press).
(2) Laug, E. P., and Nelson, K. W.: J. Assoc. Off. Agric. Chem., 25: 399, 1942.
(3) Moncorps, C.: Arch. f. Exper. Path. u. Pharmacol., 155: 51, 1930.
(4) Cole, H. N., Schreiber, N., and Sollmann, T.: Arch. Dermat. and Syphilol., 21: 372, 1930.
(5) Duemling, W. W.: Arch. Dermat. and Syphilol., 43: 264, 1941.
(6) Menschel, H.: Biochem. Ztschr., 137: 193, 1923.

DRUG ACTION UPON MYOCARDIAL EPINEPHRINE-SYMPATHIN CONCENTRATION AND HEART RATE (NITROGLYCERINE, PAPAVERINE, PRISCOL, DIBENAMINE HYDROCHLORIDE)[1]

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Received for publication October 4, 1946

Acceleration of heart action is usually due to a preponderance of cardiac sympathetic tone over cardiac vagal tone, resulting either from an absolute increase of the former, as exemplified by the action of epinephrine, or from a decrease of the latter, as exemplified by the action of atropine, or from a combination of both.

The immediate effects upon the heart of injected or secreted epinephrine and of stimulation of the cardiac sympathetic nerves are identical in so far as stimulation of the cardiac sympathetic neurones gives rise to discharges of epinephrine from their terminals into the myocardial effector cells (O. Loewi (1), Cannon and Lissák (2), Raab and Humphreys (3a)). Regardless of its origin in either the adrenal medulla or in sympathetic neurones, epinephrine seems to be transformed within the myocardium into other epinephrine-like compounds with an intact catechol nucleus (sympathin) (Cannon and Rosenblueth (4), Raab and Humphreys (3a)). However, in the case of medullary epinephrine discharges or injection of epinephrine, the accompanying rise of systemic blood pressure is likely to elicit simultaneous central stimulation of the vagus via the carotid and aortic pressoreceptor mechanism which may entirely over-shadow the heart-accelerating effect of epinephrine per se. On the other hand, intense faradic stimulation of the cardiac sympathetic nerves may convey unintended stimuli to the cardiac vagus through connecting sympathetic fibres.

Acetylcholine elicits discharges of epinephrine from both the adrenal medulla and sympathetic neurones (Feldberg, Minz and Tsudzumura (5)) and thus produces cardiac acceleration if its vagus stimulating effect is abolished through atropine (Danielopolu and Crivez (6)).

Stimulation of the splanchnic nerves leads to epinephrine discharges from the adrenal medulla (Dreyer (7) and others) and is comparable to injection of epinephrine regarding its effect on the heart rate.

In the experiments described in this paper, the above named types of artificial cardiac acceleration were first investigated regarding their relationship with the amount of epinephrine and epinephrine-like material (sympathin) found in the heart muscle at the termination of the respective experimental periods. These results, as well as the recorded heart rates, were used as standards for comparison with the results obtained under analogous experimental conditions in combination with the administration of nitroglycerine, papaverine, priscol and dibenamine hydrochloride.

[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

The purpose of these experiments was to find indications of a possible antagonistic effect of the two first-named drugs against epinephrine-sympathin on the heart and of a definite "sympatholytic" effect of priscol and dibenamine hydrochloride, the "adrenolytic" action of which had been established in previous investigations (Hartmann and Isler (8), Nickerson (9), Raab and Humphreys (3b)).

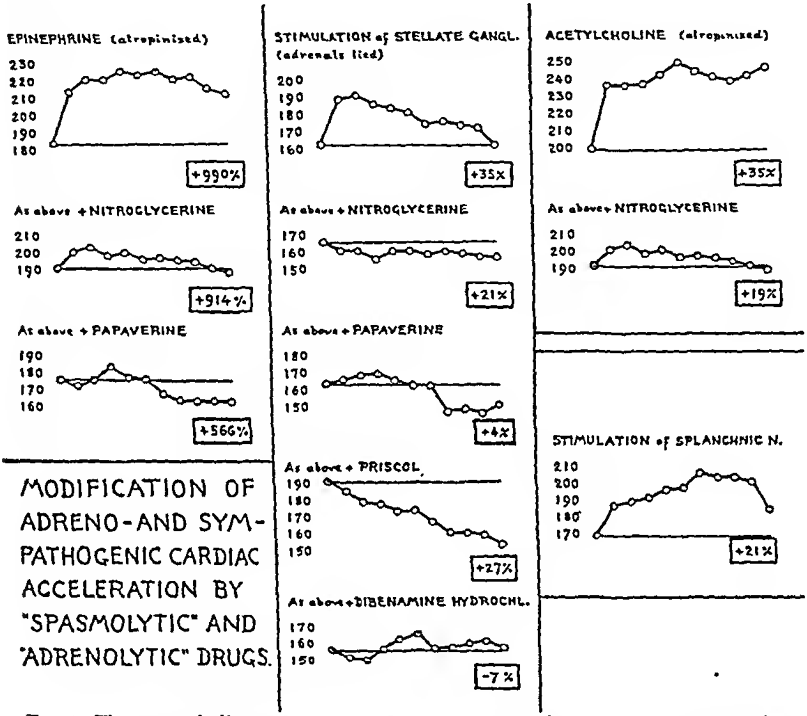

FIG. 1. The curves indicate the average heart rates per minute of 7 to 10 cats in the respective experimental groups.

The figures in squares indicate the average deviations of the myocardial concentration of epinephrine plus epinephrine-like material (sympathin), found at the end of the respective experimental periods, as compared with the average standard value found in 15 control cats.

The dosages and mode of administration of the various drugs are given in tables 2 to 12.

METHODS. All experiments were carried out in cats under nembutal anesthesia and with artificial respiration. Animals intended for epinephrine and acetylcholine injection were first atropinized (1.5–2 mgm./kgm., i.v.). In cats intended for stimulation of the stellate ganglia, the adrenal glands were first tied off. In all cases stabilization of the heart rate was awaited for about 15 minutes before the tests were begun. The heart rate was determined by palpation through an opening in the chest.

Faradic stimulations were carried out with a Harvard inductorium, connected to alternating current by means of a transformer and a resistor and with shielded Harvard electrodes. The latter were attached to both stellate ganglia (except in cats No. 23-27 where only the left ganglion was stimulated) and the ganglia were severed from the tho-

racic trunk. In another group of experiments the electrodes were attached to the splanchnic nerves on both sides.

With only a few exceptions all drugs were injected intravenously over periods of ten minutes during which the heart heats were counted each minute. Usually, one tenth of the total dose of each drug was injected during the first half of each minute and the heart rate was counted during the second half. Priscol was injected in one dose before stimulation of the stallate ganglia.

At the end of the experimental periods the hearts were excised and immediately subjected to chemical analysis for epinephrine and related compounds with the method of Shaw (10) as modified and described by one of us (Raab (11)). The colorimetric readings obtained with this method indicate the amount of sympathomimetic catechol compounds present in the tissues examined. They are expressed in color units, each of which corresponds to 0.001 gamma of epinephrine, and agree satisfactorily with quantitative biological

TABLE 1

Controls: cats in nembutal anesthesia

CAT NO.	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
	color units/gm.	
1	1,451	1.18
2	1,700	1.14
3	1,590	1.09
4	1,496	1.03
5	1,377	1.12
6	1,308	1.00
7	1,544	1.07
8	1,600	1.18
9	1,585	1.01
10	1,629	1.00
11	1,029	1.18
12	1,339	1.05
13	1,824	1.06
14	1,335	1.11
15	931	1.45
Average................	1,449	1.11

tests (3a). Whether the colorimetric readings are due to a prevailing presence of epinephrine proper or of other epinephrine-like catechol compounds (sympathin) can be estimated from the "denominator of the specific ratio" (d.s.r.). A d.s.r. near or above 2.00 indicates pure epinephrine, a d.s.r. near 1.0 indicates the prevalence of other epinephrine-like compounds.

RESULTS. Injection of epinephrine in atropinized cats (tab. 2, fig. 1) was followed by the usual massive myocardial accumulation of chromogenic epinephrine-like material (+990%) with a d.s.r. of 1.47, suggesting its only partial identity with epinephrine. The average increase of the heart rate was 40 beats per minute.

Intense stimulation of one or both stellate ganglia (tab. 3, fig. 1) elicited an initial sharp rise of the average heart rate which was followed by a gradual return toward normal. The average increase of the heart rate per minute was 20 beats

and the average increase of epinephrine-like compounds in the myocardium was 35% with a d.s.r. of 1.05.

TABLE 2

Injection of epinephrine (i.v.; cats atropinized)

CAT NO.	DOSE	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
	mgm. per kgm.			*color units/gm.*	
16	1.0	188	+37	11,622	1.57
17	1.0	160	+57	4,275	1.46
18	1.0	186	+68	7,693	1.59
19	1.0	200	+46	6,737	1.68
20	5.0	190	+43	21,622	?
21	5.0	196	+25	39,109	1.33
22	5.0	220	+4	19,533	1.20
Average.....	2.7	191	+40	15,799	1.47
Difference....................................				+990%	

TABLE 3

Faradic stimulation of stellate ganglia (adrenals tied)*

CAT NO.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
			color units/gm.	
23	144	+54	1,721	1.00
24	150	+9	1,882	1.09
25	172	+24	1,451	1.01
26	162	+30	2,080	?
27	200	+9	1,851	1.00
28	168	+29	2,613	1.04
29	154	+16	2,183	1.14
30	140	+52	1,707	0.99
31	162	+17	2,459	1.07
32	194	−40†	1,586	1.09
Average.......	165	+20	1,953	1.05
Difference......			+35%	

* Coil distance diminishing from 7 to ½ cm.

† This animal died after a temporary acceleration of the heart rate which was followed by increasing bradycardia.

Injection of acetylcholine in atropinized cats (tab. 4, fig. 1) gave rise to an average cardiac acceleration of 42 beats per minute. The increase of myocardial epinephrine-like material was 35% above the normal average with a d.s.r. of 1.06.

The effect of splanchnic stimulation upon heart rate and myocardial accumula-

tion of epinephrine-like compounds (tab. 5, fig. 1) was similar to that of acetylcholine injection.

TABLE 4

Acetylcholine injection (intravenous; cats atropinized)*

CAT NO	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN	EPINEPHRINE LIKE MATERIAL IN HEART	D S R.
			color units/gm.	
33	200	+51	2,164	1.09
34	150	+66	1,477	1.08
35	198	+32	2,051	1.21
36	214	+38	2,487	1.00
37	220	+31	2,087	1.08
38	192	+42	1,418	1.00
39	232	+30	2,349	1.13
40	184	+41	1,864	1.00
41	220	+34	1,849	1.00
42	208	+53	1,777	1.00
Average	202	+42	1,952	1.06
Difference			+35%	

* 10 mgm./kgm.

TABLE 5

*Stimulation of splanchnic nerves**

CAT NO	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN	EPINEPHRINE-LIKE MATERIAL IN HEART	D S R
			color units/gm	
43	162	+4	1,739	?
44	136	+15	1,784	1 00
45	160	+38	1,753	1.04
46	184	+17	1,960	1 00
47	182	+27	1,958	1 04
48	150	+6	1,601	1 27
49	168	+33	2,108	1.00
50	212	+23	1,585	1.11
51	150	+21	1,583	1 00
52	216	+78	1,520	1.42
Average	172	+26	1,759	1.10
Difference.			+21%	

* Coil distance diminishing from 7 to ½ cm.

Nitroglycerine exerted in most instances a markedly weakening effect on the cardiac acceleration induced by epinephrine (tab. 6, fig. 1) and by acetylcholine

(tab. 7, fig. 1). The effect of faradic stimulation of the stellate ganglia was even converted into a retardation of the average heart rate (tab. 8, fig. 1). The average accumulation of epinephrine-like substances in the heart muscle was

TABLE 6

Epinephrine plus Nitroglycerine (i.v.) (cats atropinized)*

CAT NO.	EPINEPHRINE DOSE	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
	mgm./kgm.			*color units/gm.*	
53	1.0	182	+2	10,082	1.36
54	1.0	186	+2	7,834	1.72
55	1.0	168	+30	10,190	2.85
56	1.0	176	+5	8,305	1.42
57	5.0	218	−13	21,517	1.48
58	5.0	212	+4	21,918	1.28
59	5.0	196	−5	22,982	1.33
Average....	2.7	191	+4	14,689	1.62
Difference.				+914%	

* 1 mgm./kgm.

TABLE 7

Acetylcholine (i.v.) plus Nitroglycerine (i.v.) (cats atropinized)*

CAT NO	DOSE OF NITROGL.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
	mgm./kgm.			*color units/kgm.*	
60	0.3	220	−2	1,625	0.98
61	0.3	225	−13	1,828	1.12
62	0.3	196	+3	1,514	1.02
63	0.6	172	+33	1,771	1.00
64	1.0	172	+16	1,623	1.00
65	0.6	188	+7	2,058	0.99
66	0.6	192	+11	1,451	0.98
67	1.0	188	+23	1,922	1.07
68	1.0	220	−11	1,518	0.96
69	1.2	208	−11	1,929	1.08
Average .		199	+6	1,724	1.02
Difference.				+19%	

* 10 mgm./kgm.

somewhat smaller than normal in all three experimental groups with nitroglycerine but the differences were only moderate. The d.s.r. was not altered significantly.

Papaverine practically abolished the cardiac acceleration induced by both

epinephrine (tab. 9, fig. 1) and by stimulation of the stellate ganglia (tab. 10, fig. 1) and turned it gradually into a retardation in most instances although in a

TABLE 8

Faradic stimulation of stellate ganglia plus nitroglycerine† (i.v.) (adrenals tied)*

CAT NO.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
			color units/gm.	
70	148	+13	2,012	1.12
71	160	−15	1,568	1.13
72	166	+2	2,026	1.35
73	162	−13	1,655	1.10
74	162	−29	2,134	?
75	182	−49	1,460	1.07
76	180	−13	1,824	1.04
77	168	+14	1,429	1.22
78	192	+4	1,342	0.96
79	160	+21	2,019	0.96
Average ..	168	−7	1,747	1.11
Difference. ...			+21%	

* Coil distance ½ cm.
† 1 mgm./kgm.

TABLE 9

Epinephrine plus Papaverine (i.v.) (cats atropinized)*

CAT NO	EPINEPHRINE DOSE	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN	EPINEPHRINE-LIKE MATERIAL IN HEART	D.S.R.
	mgm./kgm.			*color units/gm.*	
80	1.0	188	−13	11,373	2.13
81	1.0	180	−24	13,571	1.60
82	1.0	164	+7	12,129	?
83	1.0	192	−11	5,007	1.89
84	5.0	184	±0	3,706	3.00
85	5.0	170	−5	9,955	1.62
86	5.0	168	−7	11,806	1.81
Average ..	2.7	178	−8	9,649	2.01
Difference.				+566%	

* 5 mgm./kgm.

few experiments a certain degree of acceleration was maintained. The accumulation of epinephrine-like compounds in the heart after injection of epinephrine was markedly diminished through papaverine (+566% instead of +990%). The average d.s.r. was high: 2.01. After stimulation of the stellate ganglia the

TABLE 10

Faradic stimulation of stellate ganglia plus papaverine† (i.v.) (adrenals tied)*

CAT NO	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D S.R.
			color units/gm.	
87	164	−1	1,755	1.21
88	180	+1	1,075	1.09
89	168	+4	1,711	1.16
90	176	−36	1,043	1.18
91	148	+27	743	1.05
92	164	+9	1,679	0.99
93	174	−5	2,169	0.98
94	148	+2	1,501	0.98
95	172	−37	1,960	0.98
96	168	+18	1,422	1.42
Average	166	−2	1,506	1.10
Difference. .		.	+4%	

* Coil distance ½ cm.
† 5 mgm./kgm.

TABLE 11

Faradic stimulation of stellate ganglia plus Priscol† (i.v.) (adrenals tied)*

CAT NO.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D S.R.
			color units/gm.	
97	108	−34	2,069	1.00
98	228	−51	1,760	1.03
99	220	−6	1,857	1.00
100	212	−16	2,441	1.12
101	170	+8	2,000	1.02
102	186	−70	1,711	1.14
103	220	−15	1,435	1.00
104	210	−15	1,806	1.06
105	200	+19	1,420	1.20
Average .	195	−20	1,833	
Difference.		....	+27%	

* Coil distance ½ cm.
† 10 mgm /kgm. 35 minutes before stimulation of the ganglia.

accumulation of epinephrine-like material in the myocardium was almost abolished: only +4%.

Priscol[2] exerted a markedly retarding influence on the heart rate during stimu-

[2] Priscol was kindly supplied by Dr. Ernest Oppenheimer of Ciba.

lation of the stellate ganglia (tab. 11, fig. 1) although the myocardial accumulation of epinephrine-like material was only slightly diminished.

The effects of dibenamine hydrochloride[3] on the heart rate were somewhat irregular but the average curve (tab. 12, fig. 1) showed a marked diminution of the cardiac acceleration through stimulation of the stellate ganglia. The concentration of epinephrine-like substances in the heart muscle was even slightly below normal.

DISCUSSION. In the above described experiments all cardiac accelerations which were induced by adreno-sympathetic interference, namely, injection of epinephrine, electrical stimulation of the stellate ganglia or splanchnic nerves, and injection of acetylcholine in atropinized cats, were accompanied by an

TABLE 12

Faradic stimulation of the stellate ganglia plus dibenamine hydrochloride† (i.v.) (adrenals tied)*

CAT NO.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN	EPINEPHRINE LIKE MATERIAL IN HEART	D S R.
			color units/gm	
106	160	±0	1,536	1.42
107	144	−34	1,421	1.02
108	152	+23	1,113	0 99
109	168	−11	1,705	0.98
110	152	+25	1,296	1.32
111	160	−9	1,392	1 05
112	176	−14	1,429	0.97
113	158	+5	831	1.00
114	148	+13	2,054	1.04
115	172	+13	644	1.39
Average	159	+3	1,342	1.12
Difference.			−7%	

* Coil distance ½ cm.

† 10 mgm /kgm. in cats no. 106–110; 20 mgm./kgm. in cats no. 111–115.

accumulation of epinephrine and epinephrine-like catechol compounds (probably sympathin) in the myocardium. This was to be expected in view of the fact that the heart muscle is distinguished by the peculiarity of eagerly absorbing injected or secreted epinephrine (Raab (11)) and that stimulated sympathetic nerves discharge epinephrine into their effector cells where it seems to be transformed into sympathin which is probably identical with nor-epinephrine (literature see Raab and Humphreys (3a)).

The amounts of epinephrine-sympathin found in the heart muscle under the above mentioned experimental conditions varied widely, especially with varying

[3] Dibenamine hydrochloride was kindly supplied by Dr. W. Gump of Givaudan-Delawanna Chemical works.

doses of injected epinephrine, and there was no discernible quantitative relationship between the degree of increase of epinephrine-sympathin in the heart on one hand and the degree of increase of the heart rate on the other. Since, in another series of experiments (Raab and Maes (23)) cardiac sympathetic denervation and total sympathectomy were followed both by a diminution of epinephrine-sympathin in the heart muscle and by bradycardia of varying degrees, it would appear that changes of the heart rate are facilitated by any deviation of the myocardial epinephrine-sympathin concentration from its individual standard level without being quantitatively determined by the absolute size of this deviation. There are indications that the effects of the sympathomimetic amines on heart function may be due essentially to secondary alterations of the myocardial electrolyte distribution. This question is being studied at the present time in our laboratories.

TABLE 13

Modification of survival time after intraperitoneal injection of large doses of epinephrine (10 mgm./kgm.) in rats

PRETREATMENT WITH	MINUTES OF SURVIVAL			NUMBER OF ANIMALS	MORTALITY
	Minimum	Maximum	Average		
					%
Nothing	5	28	13	10	100
Nitroglycerine i p. (8 mgm /kgm.)	12	18	15	6	100
Nembutal i p (0.5 gm /kgm.)	17	37	27	5	100
Papaverine i.p (10 mgm./kgm.)	10	indefinite*	>53*	15	94

* One animal recovered completely; the longest measured period of survival was 308 minutes The majority of the papaverine-pretreated rats did not display the usual features of gasping, convulsions and pulmonary edema before death but lay motionless with slow respiration and heart action, in some cases showing increasing stiffness of the extremities.

By far the highest myocardial concentrations of epinephrine and epinephrine-like material were observed after intravenous injection of epinephrine. In fact, these concentrations were often so enormously high above what had been found to be the rapidly fatal level in other animals (rats) (Raab and Humphreys (3b)) that the survival of the epinephrine-injected cats for as long as 10 minutes seemed surprising. Since it appeared possible that the nembutal anesthesia might have exerted a certain protective effect, a number of tests with absolutely fatal doses of epinephrine (10 mgm./kgm.) were run in rats (see table 13). They showed that the time of survival was about doubled through nembutal anesthesia. Similar observations have been reported by Luisada (24) in his studies concerning the prevention of epinephrine-induced pulmonary edema through barbiturates.

An antagonistic effect of nitroglycerine against the vasopressor action of epinephrine has long been known (Cameron, 1906 (12)) and this vasodilating effect is generally interpreted as being located directly on the contractile substance (Sollmann (13)). While the heart rate in the intact human or animal

lation of the stellate ganglia (tab. 11, fig. 1) although the myocardial accumulation of epinephrine-like material was only slightly diminished.

The effects of dibenamine hydrochloride[3] on the heart rate were somewhat irregular but the average curve (tab. 12, fig. 1) showed a marked diminution of the cardiac acceleration through stimulation of the stellate ganglia. The concentration of epinephrine-like substances in the heart muscle was even slightly below normal.

Discussion. In the above described experiments all cardiac accelerations which were induced by adreno-sympathetic interference, namely, injection of epinephrine, electrical stimulation of the stellate ganglia or splanchnic nerves, and injection of acetylcholine in atropinized cats, were accompanied by an

TABLE 12

Faradic stimulation of the stellate ganglia plus dibenamine hydrochloride† (i.v.) (adrenals tied)*

CAT NO.	BASAL HEART RATE	AVERAGE DEVIATION OF HEART RATE PER MIN.	EPINEPHRINE-LIKE MATERIAL IN HEART	D S R.
			color units/gm.	
106	160	±0	1,536	1.42
107	144	−34	1,421	1.02
108	152	+23	1,113	0.99
109	168	−11	1,705	0.98
110	152	+25	1,296	1.32
111	160	−9	1,392	1.05
112	176	−14	1,429	0.97
113	158	+5	831	1.00
114	148	+13	2,054	1.04
115	172	+13	644	1.39
Average	159	+3	1,342	1.12
Difference.	.	...	−7%	

* Coil distance ½ cm.

† 10 mgm./kgm. in cats no. 106–110; 20 mgm./kgm. in cats no. 111–115.

accumulation of epinephrine and epinephrine-like catechol compounds (probably sympathin) in the myocardium. This was to be expected in view of the fact that the heart muscle is distinguished by the peculiarity of eagerly absorbing injected or secreted epinephrine (Raab (11)) and that stimulated sympathetic nerves discharge epinephrine into their effector cells where it seems to be transformed into sympathin which is probably identical with nor-epinephrine (literature see Raab and Humphreys (3a)).

The amounts of epinephrine-sympathin found in the heart muscle under the above mentioned experimental conditions varied widely, especially with varying

[3] Dibenamine hydrochloride was kindly supplied by Dr. W. Gump of Givaudan-Delawanna Chemical works.

ganglia) despite an almost unimpaired myocardial accumulation of epinephrine-like material, as demonstrated in our above described experiments, indicates a potent "sympatholytic" activity of this drug beside the previously studied "adrenolytic" one.

Surprisingly, the sympatholytic effect of dibenamine hydrochloride (dibenzyl-β-chloro-ethylamine hydrochloride), by far the most powerful adrenolytic drug in existence (Nickerson (9), Raab and Humphreys (3b)), proved less regular regarding the heart rate than expected despite a practically complete inhibition of myocardial sympathin accumulation during faradic stimulation of the stellate ganglia. The latter phenomenon in the heart of the cat differs also from the non-inhibition, even occasional enhancement, of the accumulation of injected epinephrine in the rat heart. These apparent discrepancies may be due, in part, to the fact that in the above mentioned rat experiments intervals up to many hours elapsed between the administration of dibenamine hydrochloride and the injection of epinephrine, while in the cat experiments with stimulation of the stellate ganglia the dibenamine hydrochloride was given simultaneously. Cardiac accelerations in the absence of any distinguishable increase of the myocardial epinephrine-sympathin concentration, as seen in some of the experiments with dibenamine hydrochloride, may possibly be explained by the assumption of a speeded up destruction of newly formed sympathin in the effector cells under the influence of dibenamine hydrochloride, although no such process could be demonstrated in vitro by simply mixing epinephrine and dibenamine hydrochloride.

SUMMARY

Injection of epinephrine or acetylcholine in atropinized cats, faradic stimulation of the stellate ganglia in cats with the adrenals tied, and faradic stimulation of the splanchnic nerves were found to be followed both by an accumulation of epinephrine and epinephrine-like catechol compounds (probably sympathin) in the heart muscle, and by cardiac acceleration.

Simultaneous intravenous administration of nitroglycerine, papaverine, priscol and dibenamine hydrochloride abolished those types of adreno-sympathetic cardiac acceleration against which they were tested, either partially or completely.

Nitroglycerine and priscol interfered only to a moderate extent with the accumulation of epinephrine-like material in the myocardium; papaverine inhibited it markedly; dibenamine hydrochloride prevented it completely during stimulation of the stellate ganglia. Thus, the mode of antagonistic action against adreno-sympathetic cardiac acceleration appears to be of a different nature in these drugs.

The anti-epinephrine-sympathin effects of nitroglycerine and papaverine regarding myocardial function suggest that the therapeutic action of these drugs in angina pectoris is not due to coronary dilatation alone but also to a specific counteraction against the myocardial metabolic effects of an excess influx of adreno-sympathogenic epinephrine in the heart.

organism is usually indirectly accelerated by various nitrites through intervention of the reflectory carotid and aortic pressoreceptor mechanism, the excised mammalian heart appears to be only slightly affected by moderate doses of nitrites (literature see Sollmann (13)). On the other hand, the striking diminution or abolition of adreno-sympathogenic cardiac accelerations by nitroglycerine without much alteration of the myocardial sympathin accumulation, as observed by us, seems suggestive of a specific desensitization of the heart muscle to epinephrine-sympathin through nitroglycerine, in analogy to the effects of this substance on the vascular muscles.

The traditional, though unproven belief that the symptoms of angina pectoris on effort, etc., be due to spastic coronary constrictions is based largely on the pain-relieving effect of nitrites which, in turn, is interpreted by most clinicians as consisting solely of coronary dilatation. However, the above described desensitizing effect of nitroglycerine against epinephrine-sympathin in the myocardium seems to rather support the conception of Shambaugh and Cutler (14), Eppinger and Levine (15) and Raab (16) who attribute the anginal syndrome to excessive influxes of epinephrine-sympathin into the heart muscle during exercise, emotions, etc., especially in view of the intensely anoxiating effect of epinephrine-sympathin on the heart muscle, as demonstrated by Gollwitzer-Meier, Kramer and Krüger (17) and others (literature see Raab (16)).

Papaverine too has been in use for a long time in Europe in the treatment of angina pectoris as a "spasmolytic" drug and has been recommended more recently also in this country by Katz and Elek (18). Like the nitrites it counteracts the vasopressor effect of epinephrine (Mercier and Delphaut (19)) but it also exerts some effects upon the heart: It was found to reduce the rate of the mammalian heart even after section of the vagi or atropinization or destruction of the stellate ganglia, thus, apparently, through direct action upon the myocardium (Macht (20)). Furthermore, papaverine decreases the irritability of the heart, as evidenced by the elevation of the threshold for ventricular fibrillation induced by faradic stimulation (Lindner and Katz (21), Wégria and Nickerson (22)). Our own observations with papaverine resemble those with nitroglycerine, as far as the counteraction against adreno-sympathetic cardiac acceleration is concerned, but there seems to be a difference in the mechanism of action in that papaverine interfered to a much higher degree than nitroglycerine with the myocardial accumulation of epinephrine-like material after both epinephrine-injection and faradic stimulation of the stellate ganglia. Also the time of survival after injection of large, fatal doses of epinephrine in rats was markedly prolonged by papaverine in contrast to nitroglycerine (see table 13). Prevention of epinephrine-induced pulmonary edema through papaverine has been reported by Luisada (24).

The "adrenolytic" efficacy of priscol (benzylimidazoline) in protecting the heart of the rat against otherwise fatal doses of epinephrine has been described in an earlier paper (Raab and Humphreys (3b)) where also the literature concerning the cardiac effects of this drug is discussed. The strongly heart-retarding effect of priscol during faradic stimulation of the cardiac accelerator nerves (stellate

BLOOD HEMOGLOBIN AND HEMATOCRIT RESULTS ON RATS INGESTING SODIUM FLUORIDE

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Received for publication October 9, 1946

A reduction of blood hemoglobin in rats receiving 50 ppm. of fluorine as sodium fluoride in their drinking water was observed recently in studies by Ginn and Volker (1). On the 30th, 44th, 65th and 86th days of fluorine exposure in that experiment, the average hemoglobin results for 18 rats were respectively as follows: 15.2, 14.7, 11.6 and 11.1 grams per 100 cc. Control rats maintained a level of 15.8 grams of hemoglobin per 100 cc. In a previous study with rabbits, Valjavec (2) injected a 1.0% solution of sodium fluoride intravenously daily over a period of 105 to 159 days so as to provide 10 to 30 mgm. fluorine per kgm. body weight daily. There appeared to be only a slight reduction in hemoglobin and red cell counts in these animals. Greenwood, Hewitt and Nelson (3), however, found no change in the hemoglobin and blood coagulation time resulting from sodium fluoride fed in milk to young dogs. Quantities of fluorine varied from 0.45 to 4.52 mgm. per kgm. body weight daily, determinations on the blood being made every two weeks over a period of 18 weeks.

Blood examinations in human cases exposed to the fluorine-mineral cryolite, were made by Roholm (4). Among 66 workers in cryolite factories, Roholm observed a slight reduction in the number of erythrocytes but hemoglobin values were unaffected. There were minor variations from the normal in the data for differential counts. Coagulation time was tested also, all such data being considered "within the normal boundaries" (4). In the majority of these workers some osteosclerosis due to fluorine was reported, indicating a relatively excessive fluorine exposure.

The uncertainties surrounding the relation of fluoride ingestion to blood hemoglobin and particularly the recent hemoglobin results of Ginn and Volker (1) suggested the accumulation of the data as reported in Tables I and II. Six studies of control and test rats included three strains of this species, four diets, several different periods of exposure and two levels of fluorine exposure. (See Table I.) With the exception of Experiment 6 these blood studies were one phase of other experiments planned to study fluorosis or rat dental caries. In Experiment 6 the rats received a Hoppert-Webber-Canniff diet (5) as modified by Ginn and Volker (1), i.e. Comet brand brown rice replaced the coarse corn meal in that diet. The composition of the diets in all six experiments is shown in Table III. With the exception of Experiment 4 (Table I), fluorine exposure consisted of 50 ppm. fluorine as sodium fluoride in the drinking water. The excessive fluorine content of diet 530 (Exp. 4) was due to 1.0% rock phosphate which contained approximately 3.6% fluorine.

Blood determinations were made on specified dates in Experiments 5 and 6

BIBLIOGRAPHY

(1) Loewi, O.: Pflügers Arch. f. d. ges. Physiol. 189: 239, 1921; 237: 504, 1936. Am. Scientist 33: 159, 1945.
(2) Cannon, W. B., and Lissák, K.: Am. J. Physiol. 125: 765, 1939.
(3) Raab, W., and Humphreys, R. J.: (a) Am. J. Physiol. (in press). (b) This Journal 88: 268, 1946.
(4) Cannon, W. B., and Rosenblueth, A.: Am. J. Physiol. 104: 557, 1933.
(5) Feldberg, W., Minz, B., and Tsudzumura, H.: Journ. of Physiol. 80: 15, 1933.
(6) Danielopolu, D., and Crivez, D.: Presse méd. 53: 57, 1945.
(7) Dreyer, G. P.: Am. J. Physiol. 2: 203, 1899.
(8) Hartmann, M., and Isler, H.: Arch. f. exp. Path. u. Pharmakol. 192: 141, 1939.
(9) Nickerson, M.: Federation Proceedings 5: 194, 195, 1946.
(10) Shaw, F. H.: Biochem. J. 32: 19, 1938.
(11) Raab, W.: Exp. Med. and Surg. 1: 188, 1943. Biochem. J. 37: 470, 1943.
(12) Cameron: Quoted by T. Sollmann, l.c., p. 516.
(13) Sollmann, T.: A Manual of Pharmacology, W. B. Saunders Co., Philadelphia and London, 1942.
(14) Shambaugh, P., and Cutler, E. C.: Am. Heart J. 10: 221, 1934.
(15) Eppinger, E. C., and Levine, S. A.: Proc. Soc. Exp. Biol. and Med. 31: 485, 1935.
(16) Raab, W.: Ann. Int. Med. 14: 688, 1940. J. Am. Med. Assn. 128: 249, 1945.
(17) Gollwitzer-Meier, K., Kramer, K., and Krüger, E.: Arch. f. d. ges. Physiol. 237: 639, 1936.
(18) Katz, L. N., and Elek, S. R.: J. Am. Med. Assn. 120: 434, 1942; 122: 196, 1943.
(19) Mercier, F., and Delphaut, L.: Cmpt. rend. de la soc. de biol. 111: 901, 1932.
(20) Macht, D. J.: Arch. Int. Med. 17: 786, 1916.
(21) Lindner, E., and Katz, L. N.: Am. J. Physiol. 133: 155, 1941.
(22) Wégria, R., and Nickerson, N. D.: This Journal, 76: 50, 1942.
(23) Raab, W., and Maes, J. P.: Am. J. Physiol. (in press).
(24) Luisada, A.: Exp. Med. and Surg. 1: 22, 1943.

or striated appearance in rats' incisors and frequently causes excessive incisor-elongation. In general, this fluorine exposure in rats is regarded as corresponding roughly to 5 ppm. fluorine in domestic water supplies, a fluorine exposure to which human population groups may be subjected (8).

While a fluorine anemia may occur as in Ginn and Volker's study, this effect presumably is associated with unspecified or unknown factors not duplicated in our studies. The possibility that a marginal nutritional iron deficiency would influence these results was to be considered. Diet 510, in Experiment 5, however, contained only 15 ppm. of iron and no detrimental effects of fluorine appear in the data for this experiment. In Experiments 2 and 3, it may be noted also, the rats were on experiment as long as one year. It is doubtful that a fluorine anemia

TABLE III

Percentage composition of diets

	DIET NO				
	550*	530	531	510	516
Corn meal	—	—	—	69 0	—
Corn starch	68 5	—	64 0	—	—
Corn flour	—	62 5	—	—	—
Brown rice (Comet brand)	—	—	—	—	60 0
Whole milk powder.	25 0	30 0	30 0	30.0	30 0
Yeast	5 0	3 0	3 0	—	—
Salt	0 5	1 0	1 0	1 0	1 0
Cod liver oil	1 0	2 0	2 0	—	—
Linseed meal	—	—	—	—	6 0
Alfalfa meal	—	—	—	—	3 0
$CaCO_3$	—	0 5	—	—	—
Rock-phosphate	0	1 0	—	—	—

* Had added 100 ppm Fe and 10 ppm Cu

should be anticipated under a variety of conditions such as were employed in our experiments.

SUMMARY

The relation of fluorine exposure to blood hemoglobin and hematocrit values in rats was studied in several strains of the species, using several diets and for different experimental periods. There were no differences between control and test animals to indicate any effect of fluorine on hemoglobin and hematocrit values for these rats' blood. It does not appear, according to these results, that 50 ppm. fluorine in the drinking water affects the hemoglobin concentration and total red cell volume in the blood of this species.

REFERENCES

(1) GINN, J T, AND VOLKER, J. F. Effect of cadmium and fluorine on the rat dentition. Proc Soc Exper. Biol. and Med., 57: 189–191, 1944.

(see Table II) and at the termination of Experiments 1, 2, 3, and 4 (Table I). Hemoglobin was determined by the method of Evelyn (6). Hematocrit values were obtained according to Van Allen (7).

TABLE I

Hemoglobin and hematocrit determinations for rats given fluoride in food or drinking water

	Experiment											
	1		2		3		4		5		6	
	Strain of rats											
	N.I.H.		Duke U.		Sherman		N.I.H.		N.I.H.		N.I.H.	
Number of rats	7	15	5	10	8	5	10	12	18	18	23	23
Days on experiment (days)	120	120	365	365	365	365	135	135	86	86	86	86
Fluorine added to water (ppm.)	—	50*	—	50	—	50	—	—	—	50	—	50
Fluorine added to food (ppm.)	—	—	—	—	—	—	—	360	—	—	—	—
Diet no.	550	550	550	550	550	550	531	530	510	510	516	516
Iron in diet (ppm.)	118	118	118	118	118	118	9	170	15	15	35	35
Hemoglobin (gm. per 100 ml.)	15.1	15.4	15.0	14.7	14.8	14.4	15.0	15.1	14.5	14.9	13.2	13.0
Hematocrit (volume—per cent)	46.1	44.9	44.0	44.7	44.3	45.6	44.5	44.4	33.6	33.7	40.0	40.4

* During the last 60 days, 100 ppm. of fluorine was added to the drinking water.

TABLE II

Periodic hemoglobin and hematocrit determinations for control rats vs. rats receiving 50 ppm. fluorine in drinking water

Blood analysis	Experiment 5*					Experiment 6†				
	No of rats	Hemoglobin‡		Hematocrit§		No. of rats	Hemoglobin		Hematocrit	
		Control	Fluoride	Control	Fluoride		Control	Fluoride	Control	Fluoride
1st (30th day)	18	12.1	12.0	38.4	38.9	24	10.9	11.1	39.6	39.8
2nd (44th day)	18	—	—	—	—	24	13.8	13.7	42.3	41.5
3rd (65th day)	18	13.2	12.2	42.8	42.3	22	13.5	14.6	40.7	42.1
4th (86th day)	18	14.5	14.9	33.6	33.7	23	13.2	13.0	40.0	40.4

* Diet 510 was fed in this experiment.
† Diet 516 was fed in this experiment.
‡ Grams per 100 cc. blood.
§ Volume per cent.

Inspection of the hemoglobin and hematocrit values in Tables I and II reveals no relation of these data to the fluoride exposures. It may be noted that 50 ppm. fluorine in rats' drinking water is sufficient to cause a very noticeable bleaching

EFFECT OF ANTIHISTAMINE DRUGS ON INCREASED CAPILLARY PERMEABILITY FOLLOWING INTRADERMAL INJECTIONS OF HISTAMINE, HORSE SERUM AND OTHER AGENTS IN RABBITS[1]

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Received for publication October 9, 1946

Some data and observations suggest that histamine may play a rather fundamental role in the local flare and wheal formation due to mechanical injury or to introduction into the skin of various chemical and biological substances, including antigenic agents (1–6). The slow progress in accumulating evidence to elucidate the role of histamine in wheal formation of allergic and non-allergic origin is undoubtedly due to the limitations of available chemical and biological methods for the accurate measurement of the minute amounts of histamine which are capable of producing physiological or pharmacological responses. The recent discovery of benzhydryl ethers (8–10) and α-aminopyridine derivatives (11–13) which exhibit some specificity as histamine antagonists indicates their use in diminishing or annulling responses to histamine, thus making it possible to differentiate between reactions due to histamine and those due to other causes.

The investigation reported here was undertaken to determine whether the administration of Benadryl[2] to rabbits would prevent the increased capillary permeability which follows intradermal injections of histamine and other substances, both antigenic and non-antigenic, some of which have been reported to liberate histamine (4–6). The experimental findings are especially significant in view of the effectiveness of Benadryl in the treatment of hay fever, urticaria and dermatoses (7) wherein vascular reactions to histamine may be involved. Some comparative studies were also made with N-p-methoxybenzyl-N-dimethylaminoethyl α-aminopyridine phosphate (Neoantergan or 2786 R.P.)[3] which was received during the course of our experiments. Both drugs are effective in alleviating bronchospasm occurring during anaphylactic and histamine shock in guinea pigs (8, 9, 11, 14) and will diminish or prevent the spasm of intestinal smooth muscle following administration of histamine (10, 11, 15). These drugs also diminish the hypotensive action of injected or liberated histamine in dogs (10, 11, 15, 16, 37). Neoantergan and Benadryl exert similar antihistamine actions, but the former possesses only a very weak anti-acetylcholine or atropine-like action (11, 15) and even stimulates the uterus and intestine. Neoantergan

[1] This investigation was supported, in part, by a grant from Parke, Davis and Company, Detroit, Michigan.

[2] β Dimethylaminoethyl benzhydryl ether hydrochloride was supplied through the courtesy of Parke, Davis and Company.

[3] We are indebted to Dr. Daniel Bovet, Laboratoire de Chimie Therapeutique, Institut Pasteur, 26 Rue de Docteur Roux, Paris, France, for a supply of Neoantergan which made possible our studies with this drug.

(2) VALJAVEC, M.: The blood picture in rabbits poisoned experimentally with sodium fluoride. Zeit. f. d. Ges. Exper. Medizin, 85: 382–386, 1932.

(3) GREENWOOD, D. A., HEWITT, E. A., AND NELSON, V. E.: The effects of fluorine on respiration, blood pressure, coagulation, and blood calcium and phosphorus in the dog. J. Am. Vet. Med. Assoc., 86: 28–42, 1935.

(4) ROHOLM, KAJ.: Fluorine intoxication. A clinical-hygienic study. H. K. Lewis & Co. Ltd. London, 1937.

(5) HOPPERT, C. A., WEBBER, P. A., AND CANNIFF, T. L.: The production of dental caries in rats fed an adequate diet. Science, 74: 77–78, 1931.

(6) EVELYN, K. A.: A stabilized photoelectric colorimeter with light filters. J. Biol. Chem., 115: 63–75, 1936.

(7) WINTROBE, M. M.: Clinical Hematology. Lea and Febiger, Philadelphia, 1942.

(8) DEAN, H. T.: Geographical distribution of endemic dental fluorosis (mottled enamel). Fluorine and Dental health. Publication No. 19. Am. Assoc. Adv. Sci., 1942.

hydrochloric acid or sodium hydroxide. Tests with saline were negative in all instances.

2. *Histamine diphosphate.* Concentrations of histamine[4] as low as 0.001 mgm./cc. produced positive trypan blue reactions in untreated animals. The halo staining pattern represented by a +++ or ++++ reaction was characteristic of the higher concentrations of histamine. The pale, central area of the site was apparently due to a local vasoconstriction or stasis which prevented the circulation of dye into the area. Almost without exception, Benadryl and Neoantergan completely blocked histamine in concentrations of 0.1 mgm./cc. or less. There was a significant diminution in responses to 0.5 mgm./cc. of histamine in animals treated with 10 mgm./kgm. of Benadryl and 5 mgm./kgm. of Neoantergan. Although the P value for the 5 mgm./kgm. dose of Benadryl did not indicate a significant difference in reactions to 0.5 mgm./cc. of histamine, tests in the treated animals were qualitatively less than those in the untreated. This was also true of the responses to a 1 mgm./cc. concentration of histamine since there was an almost complete absence of ++++ responses in animals treated with either of the antihistamine drugs.

The results indicated that there was a threshold concentration of histamine (0.5 mgm./cc.) which could usually be completely inhibited by Benadryl or Neoantergan, whereas with higher concentrations of histamine the effect on capillary permeability could not be completely blocked. Therefore, in order to enhance the possibility of blocking the effect of liberated histamine, all intradermal agents were used in the lowest concentrations necessary to produce positive trypan blue reactions in the untreated rabbit. These minimal effective concentrations were determined approximately for each agent.

3. *Substances known to cause liberation of histamine from tissues.* Trypsin[5] (crystalline, containing 55 per cent $MgSO_4$), snake venom[6], and staphylococcus toxin, like histamine, have been shown to give positive trypan blue tests when injected intradermally (5). These agents were of interest since the release of histamine from fixed tissue, which occurs during anaphylactic reactions, has also been demonstrated for snake venom, trypsin (19–24), and staphylococcus toxin (25) reactions. Control tests were also made with magnesium sulfate in concentrations equal to 55 per cent of the trypsin concentrations employed since the latter contained that percentage of the salt. It is obvious from both the qualitative data and the P values that the histamine antagonists did not prevent or lessen the trypan blue responses to trypsin, snake venom, and staphylococcus toxin (Burky strain Ha). Magnesium sulfate gave entirely negative results in both treated and untreated groups.

Of the substances tested only snake venom induced any delayed effects. Hemorrhage was regularly observed after 24 hours at sites injected with venom

[4] Concentrations given in terms of histamine diphosphate

[5] We are indebted to Dr. Gertrude Rodney, Parke, Davis and Company, for the crystalline trypsin employed.

[6] Crystalline venom of Crotalus adamanteus was obtained from Ross Allen's Reptile Institute, Silver Springs, Florida.

is definitely more potent than Benadryl with respect to antagonism of the bronchospastic action of administered histamine in guinea pigs, but there is not a wide difference in potency with respect to their ability to diminish the depressor effects of histamine in dogs (15). A few observations (17) suggest that Neoantergan diminishes the increased capillary permeability which follows intradermal injection of histamine in the dog.

EXPERIMENTAL The trypan blue test which is based on the rapid infiltration of intravenously injected trypan blue dye into areas of increased capillary permeability (4–6) was used to measure skin reactions Abdominal skin areas of adult albino rabbits were depilated 12–24 hours prior to the test by means of a thin paste made by adding water to a 1:1 mixture of barium sulfide (C P) and gum acacia powder Animals tested were divided into two groups, one being injected with an antihistamine drug whereas the other served as an untreated control group Benadryl was used in doses of 10 mgm./kgm. and 5 mgm./kgm., Neoantergan was used in a 5 mgm./kgm dose These doses represent, respectively, one-half, one-fourth, and one-fourth the subcutaneous dose of these drugs which produces toxic symptoms in the rabbit The antihistamine drug used was always administered subcutaneously 30 minutes before the test agents were injected intradermally into the abdominal skin in volumes of 0 2 cc by means of a tuberculin syringe and #27 gauge skin-testing needle. Injection sites were spaced approximately 6 cms apart and in order to control the possible influence of a skin site on the degree of dye infiltration, the injection site for each test substance and saline control was varied in a systematic manner from animal to animal Immediately following completion of the intradermal injections, 10 cc of a 1 per cent trypan blue solution were injected intravenously into the marginal ear vein Readings of the trypan blue reactions were made 15, 30, 45, 60, and 120 minutes following the intradermal injections Observations were also made 24 hours later for possible delayed effects, e g hemorrhage, hyperemia, and necrosis

RESULTS. The prevention or diminution of a positive trypan blue reaction by Benadryl or Neoantergan was used as the criterion for demonstrating that histamine was responsible for the increased capillary permeability to intradermal agents shown by the untreated animals Trypan blue responses were graded qualitatively as represented in the legend of table 1. The increased capillary permeability was measured in the unsensitized animals by the intensity of the staining at the 30 minute reading since dye infiltration was found to have reached a maximum at this time. The 60 minute reading was found to be more satisfactory for evaluating tests on sensitized animals In addition, the Chi Square test was applied and P values were calculated in all cases to determine whether the differences in trypan blue tests between drug-treated and untreated control groups were statistically significant For the purpose of this calculation, all positive reactions with a given concentration of test agent were grouped together regardless of qualitative differences. Since the number of experiments was small, the Yate's correction of the Chi Square formula was applied (18).

A. TESTS IN NON-SENSITIZED ANIMALS (table 1). 1 *Saline controls.* Saline was used as a solvent for all agents, pH determinations made on the solutions used by means of a Coleman pH meter yielded values ranging from pH 3.5 to pH 7.5 In order to make certain that the alkalinity or acidity of a solution was not a factor in altering capillary permeability intradermal control injections were made with saline adjusted to pH values of 3 5, 5.1, 7 0, and 8.7, by addition of

Tetracaine	0 3	31	2 ++ 5 + 5 0	3 ++ 4 + 1 0? 5 0	0 86			3 + 3 0	0 86
Codeine	10 0	10	1 ++ 1 + 1 0? 2 0	1 ++ 1 + 3 0	0 5				
Insulin	80 u /cc	14	1 + 5 0 1 0?	2 0? 5 0	1 0				
Mecholyl	10 0	2	1 0	1·0					
	5 0	2	1 0	1 0					
	1 0	9	3 0 1 ++	5 0					
	0 1	9	3 0 1 ++	5 0					
	0 01	7	3 0	4 0					
	0 001	7	3 0	4 0					

0 = negative response, no infiltration of dye into injection site, 0? = doubtful response, + = pale homogeneous blue spot, ++ = deep blue homogeneous spot, +++ = pale blue halo surrounding a light center, ++++ = deep blue halo surrounding a light center

* Numerical values indicate number of experiments giving the staining response indicated for the 30 minute reading

† At least five determinations were made at each pH for each group

‡ P values must be less than 0 05 for the results to be significant

TABLE 1

Trypan blue responses in normal rabbits

INTRADERMAL TEST SUBSTANCE (0 2 cc)	CONC	NO RABBITS	RESULTS*						
			Untreated	Benadryl (10 mgm /kgm)	P‡	Benadryl (5 mgm /kgm)	P‡	Neoantergan (5 mgm./kgm.)	P‡
	mgm /cc.								
0.9% Saline, pH 3.1, 5.1, 7.0, 8.7		80†	All 0	All 0		All 0		All 0	
Histamine diphosphate	1.0	35	11:++++ 1:+++	1:++++ 5:++ 2:+ 1:0? 3:0	0.098	2:+++ 2:++ 1:0?	0.6	1:+++ 2:++ 2:+ 1:0	0.7
	0.5	31	4:++++ 4:+++ 1:++	1:++ 3:+ 7:0	0 01	1:++ 2:+ 2:0	0.2	6:0	<0 001
	0.1	34	3:++++ 8:++	1:++ 11:0	<0.0001	2:0? 3:0	<0.001	6:0	<0.001
	0.01	15	8:++	7:0	<0.001				
	0.001	16	7:++ 1:0? 1:0	7:0					
	0.0001	13	6:0	7:0	1 0				
Trypsin	0.1	19	5:++ 1:+ 2:0?	5:++ 4:+ 2:0	0 81				
	0.05	30	5:++ 4:+ 2:0?	2:++ 5:+ 1:0? 5:0	0.3			3:++ 2:+ 1:0	0.56
$MgSO_4$	0.05	9	3:0 1:0?	5:0	1 0				
	0.025	9	3:0 1:0?	5:0	1.0				
Snake venom	0.0005	19	3:++ 1:+ 5:0	3:++ 1:0?	0.86				
Staph. toxin	Undi-luted	6	2:++ 1:0?	2:++ 1:+	1.0				
Heparin	10.0	26	1:++ 2:+ 2:0? 4:0	1:+ 10:0	0.41			6:0	0.35

the treated group. Such positive trypan blue tests were definitely less marked than those which followed similar injections in the sensitized rabbits.

In sensitized animals, Benadryl failed to induce either qualitative or quantitative differences in reactions to horse serum in dilutions of 1:1000 or less. With a dilution of 1:5000, the increase in number of negative responses in treated animals suggested that Benadryl was effective although the P value of 0.07 is not indicative of a significant effect. At higher dilutions, the incidence of reactions was low in both treated and untreated groups. This would suggest that at these dilutions the antigen itself was too weak to cause an appreciable response. An

TABLE 2

Trypan blue responses to horse serum in rabbits

INTRADERMAL TEST AGENT	NO. OF ANIMALS	NO. OF TESTS	UNTREATED†	NO. OF ANIMALS	NO. OF TESTS	BENADRYL‡ (10 MGM./KGM.)	P‡
A. Non-sensitized animals*							
Horse serum undiluted	9	9	3:++ 4:+ 2:0	10	10	1:++ 5:+ 1:0? 3:0	0.74
B. Sensitized animals*							
Saline, 0.9%	9	9	9:0	10	10	10:0	
Horse serum undiluted	9	9	3:++++ 6:++	10	10	3:++++ 2:+++ 4:++ 1:0	0.96
1:10	9	9	2:++++ 6:++ 1:+	10	10	1:++++ 2:+++ 6:++ 1:0	0.96
1:100	8	8	8:++	10	10	8:++ 2:+	1.0
1:1000	9	9	8:++ 1:+	10	10	6:++ 3:+ 1:0	0.96
1:5000	9	17	6:++ 2:+ 1:0? 8:0	8	16	2:++ 1:0? 13:0	0.07
1:10,000	9	17	3:++ 3:+ 1:0? 10:0	9	17	1:++ 1:+ 15:0	0.22
1:100,000	9	15	3:+ 12:0	10	17	1:+ 16:0	0.50

* Tabulated results are based on the staining at the 60-minute reading, and recorded as 0 to ++++ reactions as indicated by legend in table 1.

† Numerical values indicate number of experiments giving the response indicated.

‡ P values must be less than 0.05 for differences to be significant.

analysis of the data relating to all dilutions (84 tests in control animals and 90 tests in treated animals) revealed a significant increase of 16.6 per cent in the incidence of negative responses in the treated rabbits. The increased incidence of negative responses was almost entirely accounted for by a decrease in the number of ++ responses. There is some uncertainty as to whether Benadryl has a major effect on trypan blue responses since the differences noted were for the most part restricted to the one dilution of antigen (1:5000).

The wheals in sensitized animals formed by the intradermal injection of undiluted horse serum or that diluted 1:10 contrasted markedly in appearance at the end of an hour with those formed by the injection of any agents studied in the normal rabbits. The injection sites in the sensitized animals showed definite

in concentrations of 0.002 mgm./cc. and greater, although necrosis was never noted. Trypsin was used in doses as high as 1 mgm./cc., but delayed hemorrhage, edema, or necrosis never occurred.

4. *Tetracaine and codeine.* Tetracaine and codeine (26) were used in view of their wheal-producing activity in human skin, and produced positive trypan blue reactions. There were no significant differences between the results in treated and untreated animals.

5. *Heparin*[7] *and insulin*[8]. These were included as agents having no histamine-liberating capacity (5). Although some positive trypan blue responses to heparin were observed, these were comparable in the treated and untreated animals. Insulin resulted in essentially negative tests in both groups of animals.

6. *Acetyl-β-methylcholine* (*Mecholyl*). Since injected acetylcholine failed to increase capillary permeability (5) it is doubtful whether choline esters liberated from tissues account for positive trypan blue reactions. Nevertheless, it seemed desirable to extend the observations by employing Mecholyl, a choline ester more stable than acetylcholine. In view of the prolonged vasodilating action of Mecholyl, negative trypan blue tests with this agent would make possible the conclusion that marked vasodilatation does not in itself cause an increase in capillary permeability in localized skin areas under these experimental conditions.

With the exception of two responses in the same animal, Mecholyl did not cause trypan blue reactions even in concentrations as high as 10 mgm./cc. and 5 mgm./cc.

None of the agents in the concentrations used in this study caused "true" wheal formation in either treated or untreated rabbits, for there was no increase in size of the original bleb formed by the intradermal injection of 0.2 cc. of test substance. On the contrary, the original bleb tended to disappear an hour after injection or sooner, leaving the site demarcated by the spot of dye. The failure of rabbits to exhibit marked skin whealing (27) does not necessarily depreciate the value of observations relating specifically to alterations in capillary permeability in this species as measured by the dye method.

B. Tests in sensitized animals (table 2). Adult albino rabbits were sensitized by the subcutaneous injection at six day intervals of 0.5 cc. of horse serum[9]. The appearance of a positive Arthus reaction was used as an index of sensitivity. The sensitized animals were then divided into Benadryl-treated and untreated groups and were skin-tested with various dilutions of horse serum. Skin tests were also made with undiluted horse serum in normal rabbits in order to control the possible diminution by Benadryl of any permeability effects due to the horse serum itself.

Some non-sensitized animals showed increases in capillary permeability following injections of undiluted horse serum, which were not modified by Benadryl in

[7] Heparin, 110 units per mgm., Connaught Laboratories, Toronto, Canada.

[8] Iletin Insulin, Lilly, 80 units per cc.

[9] Normal horse serum containing 0.4 per cent purified cresols as preservative, supplied by Parke, Davis and Company.

involved in some minor way in these responses. No definite conclusions can be drawn regarding such a minor role for histamine since any diminution in the effect of a very small amount of histamine might not be apparent in the overall reaction. If, however, liberated histamine were one of several important additive factors responsible for a positive trypan blue test in the untreated animals, a diminished reaction might be expected in the treated animal. Our data indicate that such was not the case. The exact mechanism by which the intradermal test substances exert their action on capillaries, whether directly or indirectly through the release of a permeability factor other than histamine, is at present a matter of speculation. It is very unlikely that choline or a choline ester causes any appreciable localized change in capillary permeability since Mecholyl failed to induce positive trypan blue reactions. The same is true of acetylcholine (5), and atropine does not alter the cutaneous wheal and flare induced with allergens in human subjects (36).

Benadryl has been shown to reduce the severity of anaphylactic shock in dogs (37) and in guinea pigs (9, 14). These observations lend further support to the hypothesis that histamine is an important etiologic factor in the symptomatology of anaphylaxis. In the present study with sensitized rabbits the trypan blue response constituted the indicator of increased capillary permeability referable to the antigen-antibody reaction localized in the skin. Benadryl failed to eliminate the increased capillary permeability due to horse serum and failed to diminish such responses, except possibly with one dilution of serum (table 2, 1:5000). Therefore, the data do not provide any substantial support for the hypothesis that histamine is a major cause of the increased capillary permeability which accompanies the antigen-antibody reaction in the skin of rabbits sensitized to horse serum.

It is possible that the apparent ineffectiveness of Benadryl in diminishing the reactions to lower dilutions of horse serum might have been due to the fact that (a) the histamine liberated by the injection of larger quantities of antigen was greater than the threshold concentration of histamine, viz. 0.5 mgm./cc. against which a 10 mgm./kgm. dose of Benadryl is effective, or (b) histamine was only one of several causative factors and that suggestive evidence of a decrease in its activity becomes apparent only after dilution of the antigen had limited the effects of the other factor(s) involved. It is extremely unlikely that histamine is the sole factor responsible for the skin reaction to antigen since the intradermal injection of histamine in normal rabbits does not duplicate the cutaneous reaction shown by the sensitized animals. The hemorrhage associated with the intradermal injection of antigen in the sensitized animal seems indicative of a more marked effect on capillary permeability than can be accounted for by the action of histamine alone even in high concentration.

On the other hand, the data concerning the effect of Benadryl on trypan blue responses in sensitized rabbits does not exclude the possibility that histamine is involved. The method may have been inadequate to demonstrate the participation of histamine in the local antigen-antibody reaction, although the method was adequate to reveal significant differences in trypan blue responses with

edema with a two- to three-fold increase in size of the original wheal, whereas the sites in normal animals were characterized by the disappearance of the original intracutaneous bleb of fluid. Furthermore, six hours after injection, the sensitized animals in almost all instances showed marked hemorrhage at sites injected with undiluted horse serum and at those injected with a 1:10 dilution of horse serum. At the end of 48 hours, all sensitized animals showed a positive Arthus reaction to the undiluted horse serum, and many showed such a response to the 1:10 dilution.

Discussion. The fact that both Benadryl and Neoantergan annulled or diminished the trypan blue responses to intradermal injections of histamine in rabbits indicates that these antihistamine drugs prevent the increased capillary permeability due to histamine. A few observations recently made under similar experimental conditions reveal that Neoantergan (17) and Pyribenzamine (13, 28) have the same action in rabbits and dogs, respectively. Therefore, it seems justifiable to use these histamine antagonists as research tools for determining whether various substances increase capillary permeability by liberating histamine. Antihistamine drugs have also been reported to diminish the wheal and flare in human skin following introduction of histamine (29–33) although extravasation of dye was not used as a measure of increased capillary permeability in these experiments. Inconsistent results (29, 34) were probably referable to differences in potency and dosage of antihistamine drugs and to route and time of administration prior to skin-testing. Doses administered one or more days previous to the tests may not influence results since antihistamine drugs probably act only for several hours.

Recent evidence (24, 38) opposes the theory that trypsin toxicity is solely referable to histamine release (22). In the present experiments, the failure of both Benadryl and Neoantergan to diminish the trypan blue reactions to intradermally injected trypsin is further evidence against the view that the responses to trypsin are mediated by histamine. All of this evidence concerns pancreatic trypsin and does not exclude the possibility that some specific enzyme may be concerned with release of histamine in allergic and inflammatory conditions.

It has been postulated that liberation and action of histamine accounts for certain reactions to snake venom (19, 22, 23) and staphylococcus toxin (25), including the positive trypan blue response to intradermal injections of these substances (5). The release of histamine from tissues exposed to venom and staphylococcus toxin has been cited in support of this idea (19, 20, 22). An antihistamine drug, Antergan, has been reported to diminish the cutaneous reactions to venom (Viper Daboia) in human subjects (35). However, the ineffectiveness of Benadryl in preventing the trypan blue responses to these substances argues against the view (5) that such increases in capillary permeability are due to liberated histamine.

It may be concluded from the data presented that histamine is not a major factor in causing increased capillary permeability in unsensitized rabbits following intradermal injections of trypsin, snake venom, staphylococcus toxin, tetracaine and codeine. The question then arises as to whether or not histamine is

19. Kellaway, C. H.: Ann. Rev. Biochem., 8: 541, 1939.
20. Trethewie, E. R.: Australian J. Exper. Biol. and M. Sc., 17: 145, 1939.
21. Rocha e Silva, M., and Essex, H. E.: Am. J. Physiol., 135: 372, 1941–42.
22. Dragstedt, C. A., and Wells, J. A.: Quart. Bull. Northwestern Univ. M. School, 18: 104, 1944.
23. Essex, H. E.: Physiol. Rev., 25: 148, 1945.
24. Wells, J. A., Morris, H. C., and Dragstedt, C. A.: Proc. Soc. Exper. Biol. and Med., 62: 209, 1946.
25. Feldberg, W., and Keogh, E. T.: J. Physiol., 90: 280, 1937.
26. Alexander, H. L., Elliott, R., and Kirchner, E.: J. Invest. Dermat., 3: 207, 1940.
27. Darsie, M. L., Perry, S. M., Rosenfeld, D., and Zaro, J. A.: Proc. Soc. Exper. Biol. and Med., 59: 278, 1945.
28. Rennick, B., Chess, D., Hays, H. W., Mathieson, D., Mayer, R. L., and Yonkman, F. F.: Federation Proceedings, 4: 133, 1945.
29. Friedlaender, S., and Feinberg, S. M.: J. Allergy, 17: 129, 1946.
30. Parrot, J. L.: Presse med., 50: 771, 1942.
31. Sciclounoff, F., and Juinet, R.: Rev. med. de la Suisse Rom., 63: 570, 1943.
32. Warembourg, H., Linquette, and Machon: Compt. rend. Soc. de biol., 138: 417, 1944.
33. Elias, H., and McGavack, T. H.: Proc. Soc. Exper. Biol. and Med., 61: 133, 1945.
34. Staub, A. M.: Ann. Inst. Pasteur, 63: 400, 485, 1939.
35. Parrot, J. L.: Compt. rend. Soc. de biol., 136: 715, 1942.
36. Tuft, L., and Brodsky, M. L.: J. Allergy, 7: 238, 1936.
37. Wells, J. A., Morris, H. C., and Dragstedt, C. A.: Proc. Soc. Exper. Biol. and Med., 61: 104, 1946.
38. Taonon, H. J.: J. Clin. Invest., 24: 1, 1945.

graded doses of histamine and the effects of antihistamine drugs thereon. Since undiluted horse serum sometimes produced trypan blue responses in non-sensitized rabbits which were unaffected by treatment with Benadryl, there exists the possibility that the response in sensitized rabbits to serum, even though diluted, was partially due to a factor unrelated to an antigen-antibody reaction.

SUMMARY AND CONCLUSIONS

1. As shown by the trypan blue test in the rabbit, Benadryl and Neoantergan prevent or diminish the action of injected histamine in increasing capillary permeability.

2. In contrast to results with histamine, appropriate concentrations of intradermally injected horse serum, trypsin, snake venom, staphylococcus toxin, tetracaine, codeine, and heparin cause positive trypan blue reactions which are not modified by Benadryl. Therefore, liberated histamine is not a major causative factor in such instances of increased capillary permeability.

3. Trypan blue responses induced in sensitized rabbits by intracutaneous injections of horse serum were not sufficiently altered by Benadryl to provide substantial support for the hypothesis that histamine plays a prominent role in the increased capillary permeability which accompanies the antigen-antibody reaction. The involvement of histamine was not excluded.

4. Marked differences exist between the cutaneous reaction to histamine in non-sensitized rabbits and that to horse serum in sensitized rabbits.

REFERENCES

1. Lewis, Sir Thomas: The Blood Vessels of the Human Skin. London, Shaw and Shaw, 1927.
2. Krogh, August: The Anatomy and Physiology of Capillaries. Revised and Enlarged Edition. New Haven, Yale University Press, 1930.
3. Crammer, J. L., and Hele, M. P.: Nature, **154**: 18, 1944.
4. Ramsdell, S. G.: J. Immunol., **15**: 305, 1928.
5. Rocha e Silva, M., and Dragstedt, C. A.: This Journal, **73**: 405, 1941.
6. Rigdon, R. H., and Haynes, A.: J. Lab. and Clin. Med., **27**: 598, 1942.
7. Various Authors: Symposium on Benadryl. Proc. Staff Meet., Mayo Clinic, **20**: 417, 1945.
8. Loew, E. R., Kaiser, M. E., and Moore, V.: This Journal, **83**: 120, 1945.
9. Loew, E. R., and Kaiser, M. E.: Proc. Soc. Exper. Biol. and Med., **58**: 238, 1945.
10. Loew, E. R., MacMillan, R., and Kaiser, M. E.: This Journal **85**: 229, 1946.
11. Bovet, D., and Walthert, F.: Ann. pharm. franc., **2**: (suppl't to No. 4): 1–43, 1944.
12. Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Sci., **102**: 93, 1945.
13. Mayer, R. L.: J. Allergy, **17**: 153, 1946.
14. Friedlaender, A., Feinberg, S. M., and Feinberg, A. R.: Proc. Soc. Exper. Biol. and Med., **62**: 65, 1946.
15. Sherrod, T. R., Schloemer, H. F., and Loew, E. R.: Federation Proceedings, **5**: 202, 1946.
16. Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, C. A.: This Journal, **85**: 122, 1945.
17. Bonvallet, M., and Decourt, P.: Compt. rend. Soc. de biol., **138**: 305, 1944.
18. Hill, A. B.: Principles of Medical Statistics. Second Edition, London, The Lancet Limited, 1939.

reason that we assayed the amphetamine-like substance excreted by our subjects during the periods when they received placebos. These values were assumed to represent the basal output of "amine" for each subject and are presented along

TABLE I

Output of unknown amine during control periods

SUBJECT NO.	AV. DAILY AMINE OUTPUT	STANDARD DEVIATION
	mgm.	
1	3.9	±3.14
2	2.84	±1.39
3	2.23	±1.61
4	2.05	±1.37
5	2.53	±1.43
6	2.77	±2.00
7	2.53	±1.61
8	2.12	±1.25
9	2.44	±1.72
10	2.97	±1.28

TABLE II

Urinary excretion of administered amphetamine, in per cent

SUBJ.	DAILY DOSE	WEEK							
		1	2	3	4	5	6	7	8
1	30 mg.	49.5	47.6	62.3	55.1	41.0	52.4	50.7	40.1
2	dl	44.3	48.2	53.9	53.9	51.8	54.8	50.1	52.6
3		27.8	38.3	39.4	40.6	44.6	44.9	44.9	34.6
4		37.3	35.0	31.8	33.5	43.3	43.3	37.8	43.0
5		44.4	44.8	55.8	50.7	52.2	52.2	52.2	43.3
Mean..	dl	40.6	42.8	48.6	46.8	46.6	49.5	47.1	42.7
6*	15 mg.								
7	d	50.0	46.6	46.9	44.8	40.1	47.2	49.0	48.5
8		44.4	57.7	49.0	48.0	49.0	51.6	55.8	45.2
9		33.2	44.4	40.6	37.6	38.4	37.3	43.4	45.5
10		58.0	59.6	52.7	47.2	44.0	52.2	59.8	37.9
Mean ...	d	46.4	52.1	47.3	44.4	42.9	49.1	52.0	44.3
Mean, group		43.2	46.9	48.0	45.7	44.9	48.4	49.3	43.4

* The record of subject 6 has been omitted because he was ill and off medication during the fourth and fifth weeks of the treatment period.

with their standard deviations in Table I. Even with a constant diet, the excretion of the unknown "amine", though of low magnitude, was quite variable. It was also apparent in the daily records that the random fluctuations in the "amine" output occurred independently in the different subjects.

THE EXCRETION OF AMPHETAMINE[1]

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Received for publication October 25, 1946

INTRODUCTION

Certain information about the metabolism of amphetamine in the body has prompted this study. Beyer and Skinner (1), studying 15 human subjects found that slightly less than 50 per cent of a 20 or 30 mgm. dose was excreted in the urine within 48 hours. They also reported that in general, the amount of the drug excreted varied with the corresponding urine volume. Jacobsen and Gad (2), studying 4 human subjects and using doses of 20 mgm., found the drug to be excreted in the urine as long as 3 days after a single administration and during this period recovered 20–50 per cent of the administered quantity. Richter (3), using an unstated number of subjects and doses of 20 mgm., reported that 70 per cent was excreted in the urine in 48 hours.

The liver apparently changes the drug to some unknown compound. This has been demonstrated by the results of Beyer and Skinner (1) and of Jacobsen and Gad (2). The former recovered almost all of a dose of the drug in the urine of dogs with experimentally produced hepatic damage. The latter found a significant loss of the drug when it was perfused through the liver of a rabbit and a marked increase in its urinary excretion by hepatectomized rabbits. According to Beyer (4) the administration of ascorbic acid in conjunction with the drug decreases the urinary excretion of the drug.

Beyer indicates that after 3 or 4 days of administration in dogs, the urinary output of the drug becomes relatively constant and his data show this to persist for a period of 20 days when the daily dose (10 mgm.) is kept constant. Jacobsen and Gad (2) gave 5 mgm. of amphetamine to one subject daily for 30 days and found the urinary output to remain constant.

The present experiments were undertaken not only to confirm the findings of Jacobsen and Gad but to extend them to include a larger series and to employ more commonly used quantities of the drug. It was expected that changes in the metabolism of the drug, if they occurred, might be related to the development of tolerance, or of undesirable side effects of amphetamine.

Experimental results. *The control "amine" excretion.* During preliminary studies we learned that none of the tests described in the literature were specific for amphetamine. Almost every urine assayed from subjects who had never taken amphetamine gave positive values for "amine" content, some equivalent to as much as 5 mgm. of amphetamine. Jacobsen and Gad (2) had the same experience but minimized and made fairly constant the excretion of this unknown "amine or amines" by a diet exclusively of bread and butter. It was for this

[1] Aided in part by a grant from the Smith, Kline and French Laboratories.

TABLE III

Blood levels of ascorbic acid

SUBJ.	CONTROL*	2 DAYS ℞	16 DAYS ℞
	mgm. %	*mgm. %*	*mgm. %*
1	0.95	1.0	0.91
2	1.24, 1.45	1.23	1.35
3	1.12, 0.98	0.9	1.07
4	0.84, 1.05		1.13
5	0.55, 1.03	0.93	1.00
6	1.01, 1.27	0.95	1.41
7	0.87, 1.13	1.13	1.13
8	0.89	1.04	0.87
9	1.07, 1.47	1.05	1.10
10	0.94, 0.86	0.95	0.87
Average............	1.03	1.02	1.08

* Two separate determinations made during the control period, in eight of the subjects.

SUMMARY

The data reveal that when 30 mgm. of dl-amphetamine (Benzedrine) or 15 mgm. of d-amphetamine (Dexedrine) were given daily to 10 human subjects for 56 consecutive days, the daily urinary excretion of the drugs remained essentially constant. These results imply that the rate of catabolism of the drug (presumably by the liver) remained constant, and that the development of tolerance cannot be attributed to progressive changes in drug destruction.

Under the conditions of the experiment, there was no evidence of a diuretic effect of amphetamine and the excretion of the drug was independent of urinary volume.

Although it has been reported that ascorbic acid will decrease the urinary excretion of amphetamine by dogs, it was found that amphetamine did not reduce the blood level of ascorbic acid.

PROCEDURE. Ten healthy male medical students served as subjects for this experiment. Each was of normal body weight. The subjects were fed three meals a day by a diet kitchen operated solely for this group. They were required to eat all the food served to them without exception or extramural addition. The menu was arranged to provide a constant daily calorie, mineral, vitamin, protein, fat and carbohydrate intake. Coffee was permitted but the quantity imbibed remained constant throughout the experiment as did the smoking habit of those who indulged.

During the first two weeks no capsules were given. During the third and fourth weeks placebos were issued. Medication began the fifth week. Five subjects (no. 1 to 5) received 10 mgm. of dl-amphetamine (Benzedrine) and five (no. 6 to 10) received 5 mgm. of d-amphetamine (Dexedrine) three times a day. This medication was given one hour before each meal and was continued for 56 consecutive days. The program was continued for two more weeks during which placebos were again given. All urine was collected and each 24-hour sample was assayed for its total amphetamine content.

The assay method for the determination of amphetamine in urine developed by Beyer (4) gave inconsistent results in our hands.

The excretion of amphetamine. The daily "amine" excretion by weeks for each subject, corrected by subtracting the average control output (Table I) and expressing the remainder as percentage of the total intake is presented in Table II. These data reveal two principal facts. First, that the daily urinary excretion of the drug remained relatively constant over the entire eight week period, showing only random fluctuations. Second, that the average percentage recovery in the urine from the 30 mgm. daily dose of dl-amphetamine (45.6%) was practically the same as for the 15 mgm. daily dose of d-amphetamine (47.3%).

Inasmuch as our data included both the daily urine volume and daily amine output for each subject, it was possible to investigate both the influence of the drug on urine output and the influence of the urine output on amphetamine excretion. Figure 1 presents the average daily urine output by weeks and the average daily amphetamine output by weeks.

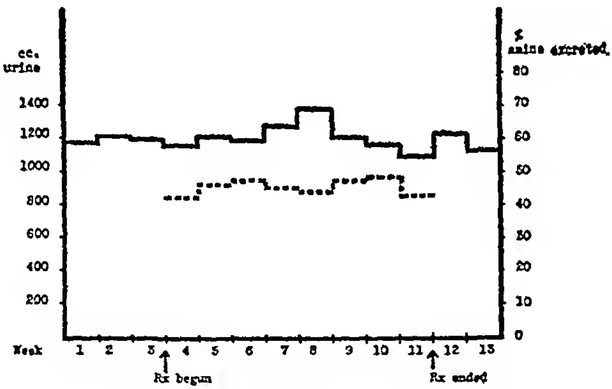

FIG. 1. AVERAGE DAILY URINE (–) AND AMINE (– – –) OUTPUT BY WEEKS

The drug had little if any effect on urinary volume, for the average daily urine volume for the entire group during the premedication period was 1210 cc. and during medication 1227 cc. Furthermore fluctuation in daily urine volume was not related to fluctuation in drug excretion (fig. 1).

Amphetamine administration and plasma vitamin C. Beyer (4) reported that ascorbic acid supplementation decreased the urinary excretion of amphetamine. On the basis of the possibility that the converse relationship might also exist we determined the ascorbic acid content of the blood of our subjects at various times during the experiment by the method of Roe and Kuether (7). It should be recalled that the vitamin C intake of our subjects remained constant during the entire experiment. The results of determinations made before, 2 days, and 16 days after the beginning of medication are presented in Table III. The failure of the medication to alter the ascorbic acid content of the blood under these circumstances is apparent.

THE EFFECT OF DIGITALIS ON THE FLUID DISTRIBUTION OF THE BODY[1]

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Received for publication October 25, 1946

Digitalis has been observed to reduce the blood volume of normal subjects and patients with both compensated and uncompensated heart disease (1, 2). The reduction in blood volume of patients with heart failure following digitalis therapy is readily understandable on the basis of improved cardiac action and lowered venous pressure. When the heart is normal, some other mechanism must be involved. Dock and Tainter (7) interpreted their studies on the reduction in cardiac output in dogs by digitalis as being due to a pooling of blood in the spleen and liver. They consider the liver especially important because of the constrictor action of digitalis on the musculature of the portal veins.

In an attempt to resolve some of the questions involving the peripheral action of digitalis, we have studied the effect of digitalis on the fluid distribution of the body as measured by the simultaneous determination of blood volume and thiocyanate "space" in dogs.

METHODS AND EXPERIMENTAL PROCEDURES. Dogs weighing 10–15 kg. were maintained in an airconditioned room. Plasma volume was determined with T1824 and "extracellular fluid" with sodium thiocyanate (8). The total amount of blood necessary for the above determinations and for a hematocrit (Van Allen) was less than 15 cc.

Two groups of animals were used. In one group plasma volume and "extracellular fluid" were determined before and four or five hours after digitalization. In some experiments the changes in these fluid compartments were followed for several days after the administration of digitalis. These dogs were given no medication other than the digitalis.

In the second group all the observations were made with the animal under light pentobarbital anesthesia. Control hematocrit, plasma volume, and thiocyanate "space" were determined, digitalis was administered, and about four hours later these observations were repeated. Anesthesia had no apparent effect on fluid distribution as far as could be determined by control observations, which are recorded at the bottom of Table II. Light anesthesia does, however, prevent vomiting that occurs in most dogs following digitalis administration.

The tincture of digitalis used contained one USP XII digitalis unit per cubic centimeter. The tincture dosage used was 0.3–0.6 cc. per kg. diluted with saline and injected intravenously. Digalen injectable was used in some cases in a dosage of 0.6 cc. per kg.

RESULTS. The effect of digitalis on plasma volume and thiocyanate "space" of normal unanesthetized dogs is recorded in Table I. Table II summarizes the results obtained on animals anesthetized with pentobarbital. The results are essentially the same in the two groups of animals. After the administration of digitalis, there is a gradual rise in the hematocrit and at the end of several hours most animals show considerable hemoconcentration. At this time most animals

[1] Aided by a grant from the Committee on Therapeutic Research of the American Medical Association.

Our method was the same as that of Jacobsen and Gad (2), except the distillation step was replaced by one in which interfering substances were adsorbed on MgO in a buffered medium, and the petroleum ether in which the color reaction was developed was dried with anhydrous sodium sulfate. This latter step is essential to consistent results as mere traces of water will give considerable color in the petroleum ether, chloroform, picric acid solution.

Our procedure is as follows: (1) Measure the 24 hour urine sample to the nearest 5 cc. (2) Place 25 cc. of urine in a 125 cc. glass-stoppered Erlenmeyer flask. (3) Add 25 cc. of McIlvaine's solution (6) buffered to pH 6.4 and 100 mgm. of magnesium oxide. (4) Shake for 5 minutes in mechanical shaker. (5) Filter using Whatman's #1 paper. (6) Add 4 cc. 10 per cent sodium hydroxide, mix thoroughly, and filter again. (7) Extract 40 cc. of this filtrate with two portions, 25 cc. each, of redistilled Skellsol "C" (Petroleum ether, B. P. 94°C.). (8). Pool these extracts and add 5 grams of anhydrous sodium sulfate powder. (9) Allow to stand overnight for the purpose of drying. (10) To 5 cc. of the dehyrated petroleum ether extract add 5 cc. of dry picric acid in chloroform solution (1 mgm. per cc.). (11) After 20 minutes read in the colorimeter (Coleman Universal Spectrophotometer) at a wave length of 420 $m\mu$ against a blank which contains 5 cc. of water-free petroleum ether and 5 cc. of picric acid in chloroform solution.

The Standard Curve for the amphetamine determination in urine was prepared as follows:

Prepare a standard solution of amphetamine in water (0.01 mgm. per cc.). Make a series of 50 cc. volumetric flasks, containing 0, 0.4, 0.8, 1.2, 1.6, 2.0 mgm. of amphetamine. Make the volume of each flask up to 50 cc. with the buffered solution (pH 6.4). Add 100 mgm. of MgO and proceed with step No. 4 as in the method described above. When the colorimeter readings are plotted against the concentrations on semi-logarithmic paper, the relationship is linear.

REFERENCES

1. Beyer, K. H., and Skinner, J.: This Journal, 66: 318, 1939.
2. Jacobsen, E., and Gad, I.: Arch. F. exper. Path. U. Pharmakol., 196: 280, 1940.
3. Richter, D.: Biochem. J., 32: 1763, 1938.
4. Beyer, K. H.: This Journal, 71: 394, 1941.
5. Gad, I.: Arch. F. exper. Path. U. Pharmakol., 196: 25, 1940.
6. McIlvaine's Standard Buffer Solutions: Handbook of Chemistry and Physics, 24th Ed., p. 944, Chemical Rubber Publishing Co., Cleveland, Ohio, 1936.
7. Roe, J. H. and Kuether, C. A.: J. Biol. Chem. 147: 399, 1943.

can be accounted for by the escape of fluid from the plasma; therefore this extra water must come from cells.

TABLE 2

Fluid distribution in anesthetized dogs before and after digitalis

DOG NO.	TIME	THIOCYANATE "SPACE"		PLASMA VOLUME		HEMATOCRIT % RBC	REMARKS
		cc./kgm.	Change	cc./kgm.	Change		
			%		%		
1-AF	9:11	280		62		39	Control
	2:11	344	23	46	26	62	4 hr. after 0.6 cc./kgm. digitalis
2-AF	10:13	319		57		33	Control
	5:40	376	18	51	11	43	6 hr. after 0.6 cc./kgm. digitalis
3-AF	9:30	327		75		31	Control
	3:12	376	15	60	20	45	6 hr. after 0.6 cc./kgm. digitalis
5-AF	9:30	274		55		42	Control
	3:30	320	17	48	13	56	6 hr. after 0.35 cc./kgm. digitalis
7-AF	9:50	370		65		25	Control
	3:50	398	8	60	8	29	6 hr. after 0.6 cc./kgm. Digalen
8-AF	9:15	315		63		41	Control
	3:30	368	17	49	22	46	6 hr. after 0.6 cc./kgm. Digalen
9-AF	9:01	261		59		48	Control
	3:43	295	13	48	19	57	6½ hr. after 0.6 cc./kgm. Digalen
10-AF	9:30	344		80		22	Control
	4:00	402	17	69	14	28	6½ hr. after 0.6 cc./kgm. digitalis
8-F	9:40	283		61		34	Control
	3:40	311	10	52	15	40	6 hr. after 0.35 cc./kgm. digitalis
Control group							
1	9:00	290		60		36	Control
	4:00	283		62		38	After 7 hr. pentobarbital anesthesia
2	10:00	349		70		28	Control
	2:00	349		70		27	After 4 hr. pentobarbital anesthesia
3	10:30	317		66		38	Control
	2:30	326		65		36	After 4 hr. pentobarbital anesthesia

There is no evidence at the present time that these extracardiac effects are concerned in the therapeutic application of cardiac glucosides.

show a significant reduction in plasma volume and an increase in "extracellular fluid" as measured by thiocyanate. These changes have been found to persist for 3 or 4 days following a single dose of digitalis. The maximum effect seems to occur within 4–8 hours; however, no attempt was made to measure maximum changes.

DISCUSSION. Many studies (3, 4, 5, 6, 7) have demonstrated the cardiac output to be reduced by digitalis. Dock and Tainter (7) ascribe this action to a storage of blood in the abdominal viscera, particularly the liver and spleen.

TABLE 1

Fluid distribution in unanesthetized dogs before and after digitalis

DOG NO.	DATE	THIOCYANATE "SPACE"		PLASMA VOLUME		REMARKS
		cc./kgm.	Change	cc./kgm.	Change	
			%		%	
1-F	4–26	316		62		Control
	4–29	340	8	44	29	4 hr. after 0.3 cc./kgm. tincture digitalis
2-F	6–5	308		64		Control
	9–22	318		62		Control
	9–23	338	8	52	17	5 hr. after 0.3 cc./kgm. tincture digitalis
3-F	4–20	—		72		Control
	5–3	270		72		Control
	5–8	320	19	52	28	4 hr. after 0.3 cc./kgm. tincture digitalis
4-F	4–18	320		67		Control
	4–24	—		67		Control
	5–6	355	11	53	21	4 hr. after 0.3 cc./kgm. tincture digitalis
	6–20	328		70		Control
	6–21	390	19	47	33	4 hr. after 0.6 cc./kgm. tincture digitalis

Aside from this effect on the liver and spleen after digitalis, a rise in the hematocrit indicates that there is some loss of fluid from the vascular system.

The increase in "extracellular fluid" was unexpected, and no explanation is available at the present time. In many experiments, digitalis caused an augmentation of 50–60 cc. per kg. This amounts to 500–800 cc. per dog increase in "extracellular fluid." Since only a small fraction of this amount of fluid could come from the vascular system, the greater proportion must come from cells.

SUMMARY

Digitalis decreases the plasma volume, increases the hematocrit and increases the "extracellular fluid." The increase in "extracellular fluid" is far greater than

THE EFFECT OF DIMETHYLAMINOETHYL BENZHYDRYL ETHER HYDROCHLORIDE (BENADRYL) UPON PERMEABILITY OF MENINGEAL CAPILLARIES

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Received for publication June 28, 1946

It has been shown previously that Benadryl causes a decrease in permeability of peripheral capillaries (1). However, a congestion of the choroid plexus not attributable to the method of sacrifice was found on post-mortem examination of dogs chronically intoxicated with this drug (2). Since the formation of the cerebro-spinal fluid is generally considered to be a process of dialysis through a semi-permeable membrane, the membrane, for the most part being the vascular endothelium of the capillary bed within the choroid plexus (3), the permeability of the meningeal capillaries as measured by fluorescein has been recorded before and after the administration of therapeutic doses of the alkamine ether in order to determine whether or not this permeability is increased in human beings.

SUBJECTS AND METHODS. Five male patients were chosen at random from the neurological service of the hospital. Three had multiple sclerosis; one, tabes dorsalis; and one, progressive muscular dystrophy. No other pathology was present aside from their basic neurological disease. Their ages varied from 31 to 45 years. The results of previous examinations of the spinal fluid were within normal limits, except in the case of the tabetic who had a colloidal gold curve of 1122100000, and a negative spinal fluid Wassermann despite a 4+ response in the blood. None of the patients had been in the hospital for less than three months. Previously established regimes were not disturbed for these experiments.

Fluorescein was used to measure the permeability of the meningeal capillaris, because it is non-toxic, and identifiable in the spinal fluid in relatively low concentration. It can diffuse readily, since it has an extremely small molecule (molecular weight 332). Previous studies in vitro have shown that changing the filtration pressure while not changing the permeability of the membrane does not influence the concentration of fluorescein in the filtrate, only the amount of filtrate, while changing the permeability of the membrane, keeping the filtration pressure constant, influences both the amount of fluid passing through the filter, and the concentration of the fluorescein in the filtrate (4). Therefore, since it is generally accepted that capillary filtration is a process of ultrafiltration, the physiologic basis of increased fluorescence in the tissues following the injection of fluorescein can be accepted as a satisfactory measure of capillary permeability.

On the morning of the examination, 10 cc. of an aqueous solution, containing

BIBLIOGRAPHY

1. Wollheim, E.: Ztschr. f. Clin. Med., 116: 296, 1931.
2. Mies, H.: Ztschr. f. Kreislouff, 23: 460, 1931.
3. Kaufmann, M.: Rev. d. Med., 4: 381, 1884.
4. Tigerstedt, C.: Skand. Arch. Physiol., 20: 115, 1908.
5. Harrison, T. R., and Leonard, B. W.: J. Clin. Invest., 3: 1, 1926.
6. Cohn, A. E., and Stewart, H. J.: J. Clin. Invest., 6: 53, 1938.
7. Dock, W., and Tainter, M. L.: J. Clin. Invest., 8: 467, 1930.
8. Gregersen, M. I. and Stewart, J. D.: Am. J. Physiol., 125: 142, 1938.

the end of the period indicated, the intravenous injection of fluorescein followed by spinal tap was repeated, using the same technique as described above.

The concentration of fluorescein in the spinal fluid was determined by the technique of Lange, Schwimmer, and Boyd (6). Under the conditions employed, the sensitivity of this method is ±2 gamma per 100 cc.

Following intravenous injection of 5 cc. of a 5% fluorescein solution to normal individuals, and to subjects similar to those used in this study, it has been shown that the content of fluorescein in the spinal fluid varies from 0 to 30 gamma per 100 cc., with an average of 7.3 gamma per 100 cc. (6). In the present experiments, 10 cc. of a 5% fluorescein solution were injected intravenously with the idea that the levels of fluorescein in the spinal fluid might be higher, thus making differences after Benadryl more easily identifiable.

Results. The level of fluorescein in the spinal fluid before the administration of Benadryl varied from 0.0 to 3.5 gamma per 100 cc. (table 1). After Benadryl these values ranged from 0.0 to 2.0 gamma per 100 cc. (table 1).

The sugar, protein, and chloride of the spinal fluid were not significantly changed by the administration of Benadryl under the conditions noted, nor was any appreciable influence upon the pressure or cellular elements of the spinal fluid observed (table 1). That Benadryl diffused readily into the spinal canal was demonstrated by the close accord between simultaneously determined values in the blood stream and spinal fluid (7). One and one-half hours after a single dose (400 mg.) of Benadryl to each of six subjects averaged levels in the blood and spinal fluid were 0.135 and 0.139 micrograms per cc. respectively. Following total dosages ranging from 8890 to 9790 mg. of Benadryl distributed over 36 days in each of 7 subjects, the averaged blood and spinal fluid levels were 0.789 and 0.769 micrograms per cc., respectively (7).

Closely correlated studies of several other factors concerned in the distribution of Benadryl through the body are summarized below, and reference made to detailed reports of such work in each instance:

(a) Blood pressure readings were made in 74 patients receiving Benadryl in daily doses ranging from 150 to 600 mg. for periods varying from 14 to 280 days (8). Forty-five who received an average of 254 mg. daily for an average of 61 days showed no change in either systolic or diastolic blood pressures (variation of ±10 were ignored). Twenty-nine patients who received an average of 289 mg. daily for an average of 68 days, developed a significant lowering of pressure. Five of the 74 patients given an average of 360 mg. of Benadryl for an average of 57 days, developed orthostatic hypotension. A single dose of 400 mg. to 14 subjects was followed by a significant lowering of systolic and diastolic pressures. Five of these subjects developed orthostatic hypotension lasting from several hours to 3 days.

(b) Capillary permeability was measured according to the method of Lange and Boyd (9) in 28 subjects before and while taking Benadryl orally. In eleven instances an average decrease of 24 per cent over control values was observed in subjects taking 150 mg. daily for three weeks or more. With a daily dose of 200 mg., changes similar in degree were observed. With doses of 300 and 400 mg.

5% fluorescein and 5% sodium bicarbonate were injected intravenously. One hour after this injection, a spinal tap was performed. The 10 cc. of spinal fluid withdrawn were used to determine the cell count; the sugar, protein and chloride content (5); the Wasserman and colloidal gold responses; and the level for fluorescein.

Each patient was then given Benadryl orally.[1] The dosage varied, depending upon the tolerance of the individual subject. Each was started on 300 mg. per

TABLE 1

Findings in the spinal fluid before and after the administration of benadryl to patients with neurologic diseases

NO. AND DIAG.	AGE	BENADRYL			FINDINGS IN THE SPINAL FLUID					Mg. per 100 cc.		
		Day of treatment	Dose begun this date	Total dose	Fluorescein	Pressure	Cells	Wassermann reaction	Colloidal gold curve	Protein	Glucose	Chloride (as NaCl)
	yrs.		*mg.*	*mg.*	*mg./100 cc.*	*mm. H_2O*	*per cu. mm.*					
1. Progressive muscular dystrophy	45	0	0	0	3.5	126	2	Neg.	Normal	41	71	730
		1	300	0								
		2	450	300								
		7	300	2550								
		8	—	3150	2.0	180	1	Neg.	Normal	35	86	712
2. Tabes dorsalis	45	0	0	0	2.5	93	3	Neg.	1122100000	43	63	715
		1	300	0								
		2	450	300								
		7	300	2850	0.0	112	1	Neg.	Normal	58	92	748
3. Multiple sclerosis	31	0	0	0	1.0	70	5	Neg.	Normal	32	65	720
		1	300	0								
		2	450	300								
		8	—	3450	2.0	130	2	Neg.	Normal	30	68	732
4. Multiple sclerosis	38	0	0	0	0.0	74	2	Neg.	Normal	23	80	730
		1	300	0								
		6	200	1500								
		7	—	1900	2.0	95	4	Neg.	Normal	26	75	712
5. Multiple sclerosis	35	0	0	0	0.0	116	2	Neg.	Normal	32	87	700
		1	300	0								
		7	—	2100	2.0	80	2	Neg.	Normal	40	82	689

day in divided doses. If well tolerated, the dose was increased the next day to 450 mg. per day, and continued at that level until the end of the experiment, unless untoward symptoms or signs appeared. In one subject, the dose had to be reduced to 200 mg. daily after 5 days because of side reactions. Three of the subjects were continued on the regime for 7 days, and 2 for 8 days. The minimum total dosage was 1900 mg. and the maximum 3450 mg. (table 1). At

[1] Generous supplies of 'Benadryl' in 50 mg. capsules have been made available through Dr. E. A. Sharp, Parke, Davis & Co., whose courtesy is herewith gratefully acknowledged.

CONCLUSIONS

1. The permeability of the meningeal capillaries to fluorescein apparently was not altered in 5 subjects, each of whom received Benadryl for one week. The total dose varied from subject to subject within a range of from 1900 to 3450 mg.

2. No recognizable changes in the spinal fluid were observed following the use of Benadryl under the above conditions.

3. If the congestion of the choroid plexus which has been observed in chronically intoxicated animals occurs in human beings following the administration of Benadryl in therapeutic doses, there is no evidence that such a change is associated with any variation in the permeability of meningeal capillaries.

4. Disturbances in the activity of the central nervous system caused by the use of Benadryl are unaccompanied by any alteration in the formation or character of the spinal fluid.

REFERENCES

1. McGavack, T. H., Elias, H., and Boyd, L. J.: J. Lab. & Clin. Med., 31: 560–574, 1946.
2. Sharp, E. A.: Personal Communication.
3. Grinker, Roy R.: Neurology, 3rd Edition, Springfield, Ill., C. C. Thomas, 1943, pp. 26–27.
4. Lange, K., Boyd, L. J.: Arch. Int. Med., 74: 175–184, 1944.
5. Todd, J. C., and Sanford, A. H.: Clin. Diag. by Lab. Methods, 10th Ed., Philadelphia, Saunders, 1943, pp. 595–601.
6. Lange, K., Schwimmer, D., and Boyd, L. J.: Amer. J. Med. Sci., 211: 611–614, 1946.
7. Gelvin, P., and McGavack, T. H.: Bull. New York M. Coll., Flower and Fifth Aven., Hosps., 9: 51, 1946.
8. McGavack, T. H., Elias, H., and Boyd, L. J.: Amer. J. Med. Sci., (In Press).
9. Lange, K., and Boyd, L. J.: Bull. New York M. Coll., Flower and Fifth Aven. Hosps., 6: 78–81, 1943.
10. Unpublished date.
11. Alving, A. S., and Miller, B. F.: Arch. Int. Med., 66: 306–318, 1940.

daily for three weeks or more in 8 and 7 subjects, respectively, the averaged decrease in permeability amounted to 36 and 42 per cent, respectively (8).

(c) The pulse rate was studied four times daily in 80 subjects and at less frequent intervals in an additional 240 individuals all of whom received dosages varying from 150 to 600 mg. of Benadryl daily for periods ranging from 14 to 414 days (10). In no instance, was a significant alteration in rate observed (values of ± 10 were ignored).

(d) In 4 subjects inulin clearance[2] was determined according to the method of Alving and Miller (11), in 4 instances before and in 7 instances after the administration of Benadryl in daily doses of 300 and 400 mg. for periods of 3 weeks or more. The averaged figure of the control periods was 113.6 and for the test periods of 109.1, respectively (10).

Discussion. It was hoped that this study would help clarify the nature of Benadryl activity upon the central nervous system. It has already been shown that Benadryl causes a congestion of the choroid plexus in chronically intoxicated dogs (2); changes in coordination when administered in large doses to human beings (1); and changes in the sensorium of normal persons as manifested by sleepiness, "grogginess" and amnesia (1). Is it possible that these manifestations depend upon an alteration in permeability of meningeal capillaries?

The present experiments answer this question only partially. They show quite clearly that the permeability of the meningeal capillaries is not increased. However, inasmuch as fluorescein penetrates into the normal spinal fluid very slightly, they do not fully preclude the possibility that a decrease in permeability could have occurred. On the other hand, the essentially identical concentration of Benadryl in blood and spinal fluid withdrawn simultaneously indicates no interference with permeability, at least to that substance. Of course it may be possible that the congestion of the choroid pleuxus in the chronically intoxicated animal is a compensatory mechanism enabling the organism to maintain a balance between the blood and the spinal fluid against a decreasing permeability.

Other factors concerned in these relationships must include variations caused by Benadryl in the vascular system. Among these are:

(a) A consistent lowering of systolic and diastolic blood pressures when given in doses of 400 mg. daily orally and frequently when smaller amounts were used.

(b) A decrease in the capillary permeability of peripheral vessels.

(c) No alteration of the pulse rate.

(d) No apparent change in glomerular filtration as measured by inulin clearance.

Since Benadryl causes no significant change in the chemistry, cytology, pressure or dynamics of the spinal fluid, it must be assumed that the effects of this drug upon the nervous system are not mediated by alterations in the spinal fluid, or its formation, but are a direct effect upon the nerve cells.

[2] Inulin clearances were carried out through the aid of Therapeutic Grant #532 from the Council on Pharmacy and Chemistry of the American Medical Association.

demonstrated to eliminate most of the effect of learning. Then each subject was required to operate the apparatus for a period of one hour. At the end of this time a placebo, consisting of a capsule containing starch, was administered, and at the end of one hour's rest the second hour's run on the machine performed.

TABLE 1

Performance in unfatigued subjects after a placebo and after 10 mgm. of amphetamine sulfate by mouth, expressed as crude scores

SUBJECT	UNFATIGUED PLACEBO	UNFATIGUED AMPHETAMINE	CHANGE
1A	89.0	89.0	0.0
1B	86.0	83.0	−3.0
2A	87.5	91.0	3.5
2B	92.0	94.0	2.0
2C	87.5	85.5	−2.0
3A	84.0	86.0	2.0
3B	80.0	84.5	4.5
3C	80.0	80.5	0.5
4A	90.0	92.5	2.5
5C	88.0	86.5	−1.5
Mean...............			0.9

Standard deviation 2.3.

TABLE 2

Performance in fatigued subjects before and after amphetamine sulfate 10 mgm. by mouth

SUBJECT	FATIGUED	FATIGUED AMPHETAMINE	CHANGE
1A	86.5	88.0	1.5
1B	79.0	84.5	5.5
2A	80.5	84.5	4.0
2B	85.0	93.0	8.0
2C	71.0	84.5	13.5
3A	78.5	81.5	3.0
3B	77.5	79.5	2.0
3C	85.0	85.5	0.5
4A	89.5	93.5	4.0
5C	87.0	84.5	−2.5
Mean...............			4.2

Standard deviation 3.9.

There was a mean decrease of 0.9% in the second run, with a standard deviation of 1.8%.

On a subsequent day the same procedure was repeated, except that the placebo was replaced by 10 mgm. of amphetamine sulphate. Table 1 shows that under these conditions the average performance of the group was slightly superior to that after the placebo, but that this difference was much less than the standard deviation.

THE EFFECT OF AMPHETAMINE SULFATE ON PERFORMANCE OF NORMAL AND FATIGUED SUBJECTS

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Received for publication August 27, 1946

It is well established that amphetamine sulfate in therapeutic doses is capable of abolishing the sense of fatigue (1). When one comes to examine the evidence for actual objective improvement in performance effected by the drug, however, one finds it to be contradictory. Finkelstein, Alpern and Gantt (2) have reviewed the literature on the subject, and presented a tabular summary of the findings of a number of investigators who used such varied indices as school performance and reaction time.

The present work is an endeavor to assess the effect of amphetamine sulfate on sustanied performance of a skilled task under normal conditions and after induced fatigue. Since one of the uses to which this drug has been put is to combat fatigue in aircraft pilots, we strove to simulate as closely as could be accomplished in the laboratory the type of activity required of pilots. To this end an apparatus of the nature of a pursuit meter was constructed, in which the subject, by means of the conventional aircraft controls, is able to control the attitude of a model airplane suspended in front of him at a distance of 6 feet at eye level. Three irregular cams actuated by an electric motor cause the airplane to deviate from level flight in three planes in space, and it is the objective of the operator to counteract this deviation by manipulation of the controls so as to maintain the model in level flight. When this alignment is maintained within certain limits inherent in the construction of the apparatus, electrical contact is completed in a circuit which incorporates a watt hour meter. Deviation of alignment beyond this fixed limit in any of the three planes breaks the circuit. Thus the percentage of time that alignment is maintained can be determined by the reading of the watt hour meter. Such readings are secured for each period of 90 seconds of operation of the apparatus, and may be totalized for any desired longer period. The limits of alignment were so chosen that no subject was able to make a perfect score, as can be seen from table 1.

In order that brief "spurts" of concentration on the task should not introduce error, each period of operation consisted of one hour of continuous operation of the device, of which only the last 10 minute period was used, nor was the subject allowed to have knowledge either of the elapsed time or of the portion of the test which would be scored. In order to determine the validity of comparing the scores of successive tests, and also of the possible suggestive effect of administration of medication, a control series of experiments was performed. The subjects, 10 in number, were medical students in the third decade of life, in good general health. Four hours' experience in operating the device was

THE ANTIDOTAL ACTION OF p-AMINOPROPIOPHENONE WITH OR WITHOUT SODIUM THIOSULFATE IN CYANIDE POISONING

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Received for publication October 14, 1946

During World War I, the French first used hydrocyanic acid shells in the battle of the Somme (1). Although modern strategists do not agree that it was an efficient weapon, the belligerent armies in World War II undoubtedly did not dismiss its possible use. Aside from rumors that the Japanese intended to use hydrocyanic acid on the Chinese, no warring nation apparently resorted to it. Several high Nazi officials, after the German capitulation, took their lives by swallowing a solution of hydrocyanic acid which was put up in vials and distributed among them.

Lt. Colonel Bodansky (2) recently revealed that at the Medical Division of Chemical Warfare Service, Edgewood Arsenal, investigations were undertaken to find suitable agents, in addition to the gas mask, that would be effective in protecting troops against hydrocyanic acid. He and his associates (3–6) reported that p-aminopropiophenone (for short, PAPP), which forms methemoglobin, as previously shown by Vandenbelt, Pfeiffer, Kaiser, and Sibert (7), could serve as a prophylactic drug against poisoning by hydrocyanic acid and cyanogen chloride in dogs and man. Their primary aim was to induce a safe level of methemoglobinemia before encountering an enemy attack with hydrocyanic acid gas.

In these laboratories, it was shown that sodium nitrite, a drug capable of forming methemoglobin, injected intravenously, could save lives of dogs poisoned with sodium cyanide (8). If, however, the nitrite were immediately followed by the intravenous injection of sodium thiosulfate, the antidotal action would be greatly potentiated. The successive administration of sodium nitrite and sodium thiosulfate can revive dogs from cyanide poisoning even at the point of respiratory failure but prior to heart stoppage. Success of this treatment in human cases has been repeatedly recorded (9).

It would be, therefore, of interest to compare the efficacy of p-aminopropiophenone and sodium nitrite, as well as that of their respective combinations with sodium thiosulfate, in the treatment of cyanide poisoning in dogs. With Colonel Oscar Bodansky's approval and his generous supply of p-aminopropiophenone, we proceeded to carry out a series of such experiments. As in the work previously reported (8), p-aminopropiophenone was injected intravenously after the subcutaneous administration of sodium cyanide in a group of unanesthetized dogs.

Since p-aminopropiophenone is sparingly soluble in water, it was dissolved in dilute hydrochloric acid (7). In our work, a 0.1 per cent solution was employed. To make a volume of 100 cc., it was necessary to dissolve 100 mg. of

Finding no significant difference in performance of unfatigued subjects after amphetamine, it was determined to investigate the effect of the drug on the same individuals after subjecting them to a standard degree of fatigue. To this end they were required to remain without sleep for a period of approximately 36 hours, at the end of which time they were given a one hour run on the coordination apparatus, and the score of the last 10 minutes of this run used as the criterion of performance after fatigue. Ideally, it would have been desirable to have determined the effect of a placebo in the fatigued state, but the procedure was sufficiently unpleasant to the subjects that it was felt that their cooperation for two such tests could not be secured. Therefore, 10 mgm. of amphetamine was administered after the first run on the machine, and after an hour's rest the second run was performed. As seen from table 2, the drug resulted in a definite improvement in performance in all but one subject, the mean increase in performance being slightly larger than the standard deviation.

If we consider the average performance of the subjects in the unfatigued state as 100%, then that of the same subjects when subjected to fatigue by deprivation of sleep was equal to 94%. This was increased to 98% by the administration of amphetamine. From this it may be concluded that amphetamine sulfate in this dosage is capable of significantly improving the performance of a skilled task when this performance has deteriorated due to fatigue, but that this improvement is not sufficient to effect a return of performance equal to that shown in the nonfatigued state.

SUMMARY

Amphetamine sulfate in a dose of 10 mgm. by mouth is incapable of significantly improving performance of a monotonous skilled task for a relatively short time unless this performance has been reduced by previously existing fatigue. In fatigued subjects significant restoration of performance, although not to pre-fatigue levels, usually results from this medication. Thus amphetamine sulfate may be a useful drug when necessity requires the performance of such tasks by fatigued individuals.

REFERENCES

1. Ivy, A. C., and Krasno, L. R.: War Med. 1: 15, 1941.
2. Finkelstein, N., Alpern, E. B., and Gantt, W. H.: Bull. Johns Hopkins Hosp. 76: 61, 1945.

ment. Compared with the combination of sodium nitrite and sodium thiosulfate, the data of which were previously published (8), the present pair appears numerically superior. However, the standard error of the LD_{50} of sodium cyanide under the nitrite-thiosulfate treatment is so large that the difference between the two combinations is statistically insignificant. The optimal dose of *p*-aminopropiophenone in the combination was found to be the same as when it was used alone, namely, 10 per cent of the LD_{50}, or 0.71 mg. per kg. Larger doses caused an unfavorable outcome. Several of our animals which received

TABLE 1

Comparison of antidotes in cyanide poisoning in dogs

ANTIDOTE	SODIUM CYANIDE	NUMBER DIED / NUMBER USED	LD_{50} OF SODIUM CYANIDE ± S.E.
	mg. per kg.		*mg. per kg.*
None	4	0/3	5.36 ± 0.28
	5	1/3	
	6	8/10	
p-Aminopropiophenone	18	0/6	
	24	3/5	23.4 ± 1.6
	30	3/3	
p-Aminopropiophenone and sodium thiosulfate	90	0/1	
	96	0/1	
	102	2/5	105.6 ± 3.2
	108	3/4	
	114	3/5	
	120	4/4	
Sodium nitrite	18	0/3	
	24	2/5	27.1 ± 3.1
	30	3/5	
Sodium nitrite and sodium thiosulfate	60	1/3	
	72	1/3	
	78	2/5	96.7 ± 23.6
	120	3/7	
	126	3/3	

small doses of sodium cyanide such as 24 mg. per kg., but 12.5 to 20 per cent of the LD_{50} of *p*-aminopropiophenone prior to thiosulfate injection, promptly died. With the nitrite-thiosulfate combination, the most suitable dose of sodium nitrite was observed to be 22.5 mg. per kg., almost 50 per cent of its LD_{50} (8). It was our general impression that cyanide-poisoned dogs were more alert and less drowsy when treated with sodium nitrite and sodium thiosulfate than with *p*-aminopropiophenone and sodium thiosulfate.

It must be realized that the treatment of our dogs was carried out under the most ideal conditions, that is, the antidotes were given by vein immediately after

p-aminopropiophenone in 10 cc. of N/10 HCl in a volumetric flask, and dilute the whole to the mark with distilled water. Sodium cyanide, the poison, was used in 4 to 10 per cent solution, depending upon the size of the dose to be administered.

As stated above, the last part of our investigation was devoted to the evaluation of the combined antidotal action against sodium cyanide of *p*-aminopropiophenone and sodium thiosulfate, successively injected by vein. A freshly prepared 50 per cent solution of the latter was employed—the dose being 1 gm. per kg., or less. Both antidotes were repeated when toxic signs recurred or persisted. In a few dogs, the amounts of methemoglobin and blood thiocyanate were followed during the process of detoxification. The methemoglobin was determined in a Beckman DU model quartz spectrophotometer; and the thiocyanate, by a micromethod developed in these laboratories (10).

RESULTS. *1. Toxicity of p-aminopropiophenone.* Vandenbelt, Pfeiffer, Kaiser, and Sibert (7) showed that dogs died at 95 per cent methemoglobinemia and that recovery might occur with 87 per cent methemoglobinemia. In our dogs the number of deaths to the number of animals at various dose levels of *p*-aminopropiophenone expressed in mg. per kg. given intravenously, was as follows: 1/5 with 5, 6/10 with 7.5, 7/10 with 10, and 5/5 with 15. The computed median lethal dose, or LD_{50}, from these data is 7.15 ± 0.89 mg. per kg. Thus, it is a highly toxic product.

2. Its antidotal action against cyanide. It was found by repeated trials that the most suitable dose of *p*-aminopropiophenone by intravenous injection to revive dogs poisoned by subcutaneous administration of sodium cyanide was 10 per cent of the LD_{50}, namely, 0.71 mg. per kg., for the first two or three doses. Larger amounts frequently lessened the chances of recovery. All subsequent doses were one-half of that amount, that is, 0.36 mg. per kg.

In table 1, it will be observed that *p*-aminopropiophenone definitely detoxified sodium cyanide in unanesthetized dogs. It saved 3 out of 5 dogs which had received the poison in the dose of 24 mg. per kg.; but none, in the dose of 30 mg. per kg. The LD_{50} of sodium cyanide with *p*-aminopropiophenone was raised from 5.36 ± 0.28 to 23.4 ± 1.6 mg. per kg.

In the same table, it will be seen that the LD_{50} of sodium cyanide with sodium nitrite was found to be 27.1 ± 3.1 mg. per kg. The crude data were those given in our previous paper (8), plus the readings on the smallest dose of cyanide recorded during the present investigation. *p*-Aminopropiophenone has a closely comparable action in detoxifying sodium cyanide to that of sodium nitrite, since the difference in LD_{50}'s is not statistically significant. Obviously, both compounds can produce the same degree of methemoglobinemia which constitutes the mechanism of detoxification.

3. Combined antidotal action of p-aminopropiophenone and sodium thiosulfate. As expected, intravenous injection of *p*-aminopropiophenone immediately followed by sodium thiosulfate showed a potentiation of antidotal action. The combination raised the LD_{50} of sodium cyanide to 105.6 ± 3.2 mg. per kg.—almost twentyfold the LD_{50} of sodium cyanide without any treat-

ment. Compared with the combination of sodium nitrite and sodium thiosulfate, the data of which were previously published (8), the present pair appears numerically superior. However, the standard error of the LD_{50} of sodium cyanide under the nitrite-thiosulfate treatment is so large that the difference between the two combinations is statistically insignificant. The optimal dose of *p*-aminopropiophenone in the combination was found to be the same as when it was used alone, namely, 10 per cent of the LD_{50}, or 0.71 mg. per kg. Larger doses caused an unfavorable outcome. Several of our animals which received

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It must be realized that the treatment of our dogs was carried out under the most ideal conditions, that is, the antidotes were given by vein immediately after

sodium cyanide was injected subcutaneously. Methemoglobinemia had occurred before the poison was completely absorbed—thus exerting, in part, a prophylactic effect. The efficacy of the *p*-aminopropiophenone-thiosulfate or nitrite-thiosulfate combination would be expected to diminish if its administration were progressively delayed.

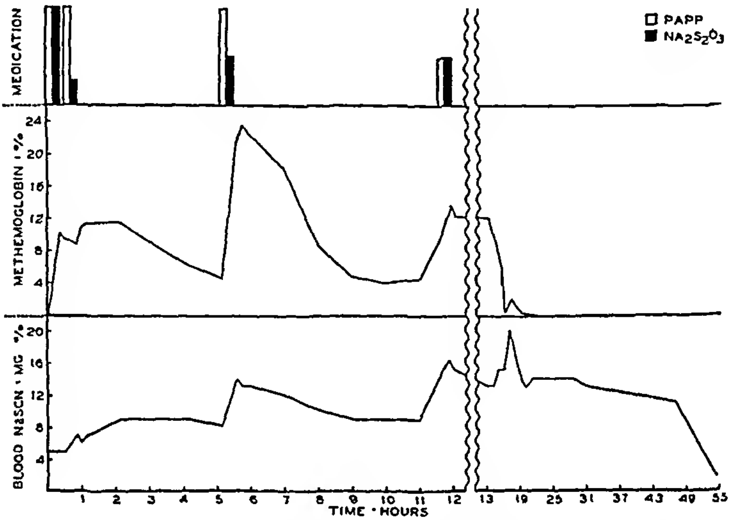
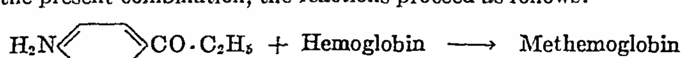


FIG. 1 DETOXIFICATION OF SODIUM CYANIDE BY *p*-AMINOPROPIOPHENONE AND SODIUM THIOSULFATE

Dog numbered 3628, female, weighing 11.1 kg., was injected subcutaneously with 96 mg. of sodium cyanide per kg. of body weight. Intravenous injection of *p*-aminopropiophenone (PAPP) in the dose of 0.71 mg. per kg., and then sodium thiosulfate ($Na_2S_2O_3$) in the dose of 1 gm. per kg., immediately followed. The dog vomited at the end of 32 minutes after cyanide injection. Treatment was resumed at the end of 37 minutes—PAPP, 0.71 mg. per kg.; and $Na_2S_2O_3$, 280 mg. per kg. An accident prevented the completion of the full dose of the latter drug, which was intended to be 1 gm. per kg. The animal again vomited at the end of 4¾ hours. Treatment was given at the end of 5 hours and 25 minutes—PAPP, 0.71 mg. per kg.; $Na_2S_2O_3$, 0.5 gm. per kg. Convulsions occurred during the injection of the antidotes, but disappeared thereafter. At the end of 11½ hours, the dog became ataxic, and the last dose of each antidote was administered—PAPP, 0.36 mg. per kg.; $Na_2S_2O_3$, 0.5 gm. per kg. A total of 34 blood samples were drawn from the external jugular vein for the measurements of methemoglobin and sodium thiocyanate during the entire course of 54¼ hours. The changes of both are shown by the curves.

The mechanism of detoxication is clearly the same in quality and in quantity. With the present combination, the reactions proceed as follows:



$$NaCN + \text{Methemoglobin} \longrightarrow \text{Cyanmethemoglobin}$$

$$Na_2S_2O_3 + NaCN \longrightarrow NaSCN + NaHSO_4$$

There is ample proof of the occurrence of three steps in detoxification. The primary formation of methemoglobin by *p*-aminopropiophenone is a matter of

certainty (7). The successful competition for the cyanide ion by methemoglobin over cytochrome oxidase has been recently elucidated *in vitro* by Albaum, Tepperman, and Bodansky (11). The conversion of the cyanide ion to sodium thiocyanate by sodium thiosulfate has been repeatedly demonstrated (12–16). The last step is achieved by the enzyme rhodanese (17–18).

The detoxifying processes can be demonstrated in experimental animals. Figure 1 shows the amounts of methemoglobin and sodium thiocyanate present in the circulating blood of a dog poisoned with sodium cyanide and successfully treated with *p*-aminopropiophenone and sodium thiosulfate. In that experiment, the methemoglobin fluctuated but never exceeded 24 per cent. Obviously, the effective concentration can stay on the safe side and need not approach anywhere near the lethal level. The formation of sodium thiocyanate, as measured in blood, steadily increased, reaching a peak of about 20 mg. per 100 cc. At the end of 54½ hours, it was still detectable to the extent of 2 mg. per 100 cc. The results from 4 other dogs showed a similar picture.

From a clinical point of view, the combination of *p*-aminopropiophenone and sodium thiosulfate deserves a trial, because its efficacy is comparable to that of sodium nitrite and sodium thiosulfate. The absence of undesirable vasomotor side-effects following intravenous injection of *p*-aminopropiophenone, as reported by Tepperman, Bodansky, and Jandorf (5), is an advantage over the use of sodium nitrite. One must realize, of course, that, weight for weight, *p*-aminopropiophenone is more toxic than sodium nitrite. Furthermore, our results in dogs clearly indicate that *p*-aminopropiophenone should be employed in proportionately much smaller doses than sodium nitrite.

SUMMARY

1. Dogs poisoned by subcutaneous injection of sodium cyanide can be saved by intravenous administration of *p*-aminopropiophenone, provided the dose of the poison is under 4 LD_{50}'s.

2. Potentiation of antidotal action against cyanide occurs when *p*-aminopropiophenone and sodium thiosulfate are successively injected by vein. The amount of sodium cyanide detoxified by this combination is about the same as that by sodium nitrite and sodium thiosulfate.

Acknowledgment. The authors are indebted to Miss Marian H. Ellaby, and Messrs. Robert C. Anderson, Harold M. Worth, E. Brown Robbins, J. Hardin Wheeler, Kenneth R. Kinningham, Davis V. Rawlings, John K. Henderson, and Francis W. Israel, for their invaluable assistance in various experiments.

BIBLIOGRAPHY

1. Prentiss, A. M.: Chemicals in War. McGraw-Hill Book Company, Inc., New York, 1st ed., 172, 1937.
2. Bodansky, O.: Science, 102: 517, 1945.
3. Jandorf, B. J., and Bodansky, O.: Federation Proc., 5: 184, 1946; J. Ind. Hyg. & Toxicol., 28: 125, 1946.
4. Tepperman, J., and Bodansky, O.: Federation Proc., 5: 104, 1946; J. Pharmacol. & Exper. Therap., 88: 287, 1946.

5. Tepperman, J., Bodansky, O., and Jandorf, B. J.: Am. J. Physiol., 146: 702, 1946.
6. Whittenberger, J. L., Wexler, J., Himmelfarb, S., and Dumke, P. R.: Federation Proc., 5: 212, 1946.
7. Vandenbelt, J. M., Pfeiffer, C., Kaiser, M., and Sibert, M.: J. Pharmacol. & Exper. Therap., 80, 31, 1944.
8. Chen, K. K., Rose, C. L., and Clowes, G. H. A.: Am. J. Med. Sc., 188: 767, 1934.
9. Chen, K. K., Rose, C. L., and Clowes, G. H. A.: J. Indiana M. A., 37: 344, 1944.
10. Anderson, R. C., and Chen, K. K.: J. Am. Pharm. A., 29: 152, 1940.
11. Albaum, H. G., Tepperman, J., and Bodansky, O.: J. Biol. Chem., 163: 641, 1946.
12. Lang, S.: Arch. f. exper. Path. u. Pharmakol., 34: 247, 1894.
13. Hebting, J.: Biochem. Ztschr., 28: 208, 1910.
14. Bodansky, M., and Levy, M. D.: Arch. Int. Med., 31: 373, 1923.
15. Bodansky, M.: J. Pharmacol. & Exper. Therap., 37: 463, 1929.
16. Smith, R. G., Mukerji, B., and Seabury, J. H.: J. Pharmacol. & Exper. Therap., 68: 351, 1940.
17. Lang, K.: Biochem. Ztschr., 259: 243, 1932.
18. Cosby, E. L., and Sumner, J. B.: Arch. Biochem., 7: 457, 1945.

A QUANTITATIVE METHOD FOR THE MEASUREMENT OF CIGARETTE SMOKE IRRITATION

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Received for publication October 28, 1946

A number of publications concerned with the evaluation of the irritant properties of cigarette smoke have appeared during the past decade. In general, the methods employed may be divided into two groups, namely, those that make use of smoke solutions prepared prior to application and those in which whole smoke is applied directly.

Of the techniques in which smoke solutions are employed, that of Mulinos and Osborne (1) is based upon the effects on the structures of the eye of the rabbit of instilling measured amounts of smoke solution into the conjunctival sac. Criteria used for evaluating irritation include visual estimation of the degree of blepharospasm produced, the amount of objection by the animal, extent of erythema, and the degree and duration of any resulting edema. Since measurements so made are subject to the vagaries of human judgment, results that represent other than gross differences are subject to justifiable dispute.

A procedure that permits of more accurate evaluation of the irritant properties of smoke solutions has more recently been described by Weatherby (2). Measured quantities of smoke solutions are injected subcutaneously into the dorsal surface of the ears of albino rabbits. Subsequently a standardized amount of trypan blue is administered intravenously. Accumulation of the dye at the sites of injection of irritant solutions was found to be proportional to the degree of irritation, color intensity measurements being made against a standard color scale. Despite the distinct advance embodied in this procedure, as regards cigarette smoke irritation, it, too, is subject to a basic criticism of all techniques making use of smoke solutions, namely, that owing to such factors as solubility and physical and chemical state, the irritant potentialities of whole smoke may not be reproduced in smoke solutions.

Of the available procedures involving the use of whole smoke, all depend upon cooperation of human subjects, and hence are not ordinarily suitable as routine procedures. Those depending upon changes in the nose, mouth, throat or larynx of man resulting from smoking (3, 4, 5) are in addition not subject to a high degree of reliability (6). That of Holck and Carlson (7) in which increase in salivary flow is used as an index of irritation, includes the factor of taste which would seem to broaden the scope of the term irritation beyond that here intended.

In an effort to obtain a method for assaying cigarette smoke irritation that would minimize the above objections, we have evolved the following procedure.

METHOD. In deriving a method for measuring cigarette smoke irritation it was felt that the following criteria must be satisfied: namely, whole smoke

must be used, the tissue exposed must represent a true mucous membrane, the results of irritation must be capable of quantitative measurement, and lastly, the method should be suitable for routine laboratory use.

The rabbit eye was selected as a convenient site for testing, since it may be easily exposed to whole smoke samples without previous surgical manipulation. Of the tissues of the rabbit eye, the membranous lining of the upper lid was found to show a graded response in the form of edema to cigarette smoke irritation. The amount of edema produced was found to be measurable by excising the membrane, drying it and calculating its water content as compared to that found for the excised membrane of the control eye.

It is recognized that this procedure has certain limitations. Only those irritants that produce edema are measured. Obviously subjective phenomena associated with irritation are not measured. Where the subjective effects are minimal as compared with the edema-producing effects this is probably unimportant. Where the subjective effects predominate the procedure is not entirely adequate. In fact, it is improbable that a single index of all manifestations of irritation can be found. Accepting this, we have worked out the following details designed to permit the quantitative measurement of the edema-producing properties of the irritants in cigarette smoke.

Male albino rabbits, weighing about 2 Kg., are morphinized (20 mg. per Kg. of morphine sulfate administered subcutaneously) to produce analgesia and to reduce struggling during subsequent handling. Twenty-five minutes after the morphine administration, 3 or 4 drops of 0.85 per cent sodium chloride solution are instilled into each eye in order that the ocular tissues may be uniformly moist at the time of exposure to smoke. A piece of thread is next attached to the lashes of each upper lid by means of scotch or adhesive tape in order that by traction on the threads the upper lids may be everted for complete and uniform exposure to smoke. The cigarette to be smoked is then inserted into a holder and sealed in place with dehydrated Karo syrup to prevent air leaks around the edges during puffing. The holder is then attached to an eye cup which in turn is attached to an automatic smoking machine of the type described by Bradford, Harlan and Hanmer (8) regulated to draw a 35 ml. puff of 2 seconds duration once a minute. The arrangement of these devices is diagramed in figure 1.

At this point either of two procedures may be followed and for convenience in future reference one will be called Procedure A, the other Procedure B.

Procedure A: Thirty minutes after administration of the morphine and in time with the puff period in the activity cycle of the smoking machine, the eye cup is pressed against a hard surface and the cigarette lit from the flame of a micro burner. This initial puff is discarded and residual smoke in the eye cup is permitted to escape. About 10 seconds before the next puff by the machine is scheduled to occur, the upper lids of both eyes are everted and the eye cup is placed over the lid and the other structures of the right eye and held firmly in place. A predetermined number of puffs (usually 3) are then drawn past the eye, the eye cup being held in place until exactly one minute after the start of the last puff desired. The retraction threads are then removed by cutting the lashes below the point of attachment.

A period of one hour (from the first puff) is allowed for edema to develop. At the end of this period the animal is killed by cerebral concussion, placed on an animal board and the upper lid of the exposed eye retracted to reveal its membraneous lining for excision. (On rare occasions it will be found that conjunctival hemorrhages occur following cerebral concussion. In such instances the animal is discarded. In our experience this occurs in approximately one per cent of the animals used.) The membrane is then carefully excised, care being taken not to

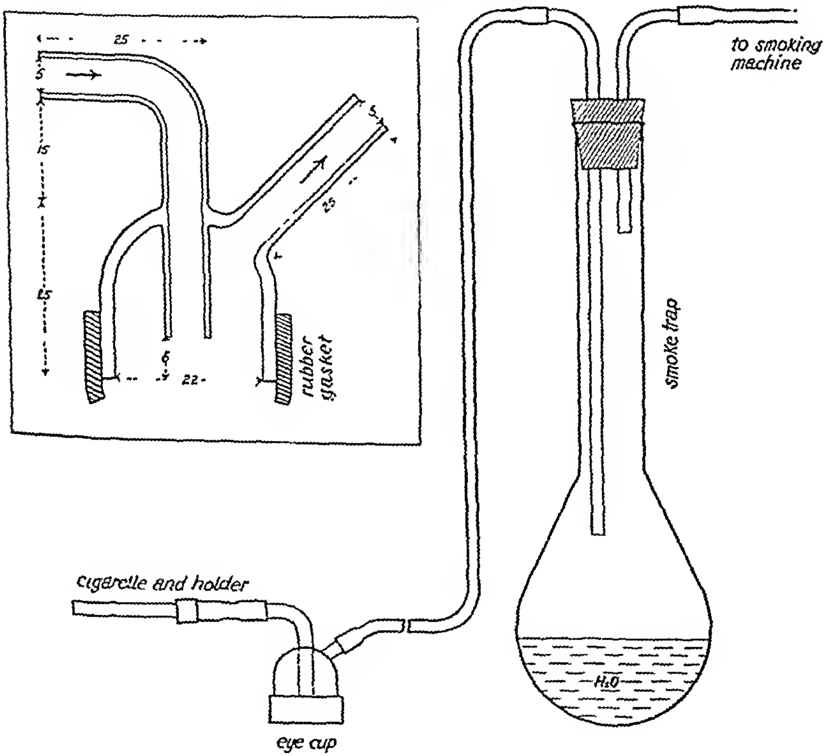

FIG 1

include the muscular tissue beneath it or the tissue of the nictitating membrane. The membrane from the control eye is similarly removed. The excised tissues are immediately placed in tared vials. Both tissues are then weighed and placed in an oven at 104°C. for 18 to 24 hours, at which time they are reweighed to obtain their dry weights.

From the wet and dry weights of the exposed and control tissues the moisture content of each is calculated. The ratio of moisture content to dry weight for each tissue is then determined. Per cent change in water content between the

two tissues may then be calculated from the ratios. In a series of determinations, the ratios serve as a basis for statistical evaluation.

Procedure B: In this procedure a control (unexposed) eye is not used as a basis of comparison. Instead, the edema-producing effects of two cigarettes are compared directly on the same animal by exposing one eye to a predetermined number of puffs from one cigarette and the other eye to an equal number of puffs from the second cigarette. Under the conditions we have employed, both cigarettes are not smoked simultaneously, smoking of the second cigarette following immediately the smoking of the first. To compensate for the difference this produces in the time for edema development, in a series of animals such comparisons are always made on even numbers of cigarettes from each lot and the sequence of smoking alternated. Also, to allow for possible inherent differences in the irritability of right eyes of rabbits as compared to left eyes, of two lots of cigarettes being compared half of each lot is tested on right eyes and half on left eyes. With the exception of these differences, Procedure B is identical with Procedure A.

Calculation of results obtained are carried out in the same way as in Procedure A.

Data pertinent to the application of the method. (a) *Normal water content of the tissues employed.* The method described is based on the assumption that the membranous linings of the upper lids of both eyes of any given rabbit initially contain equal percentages of water. To test this thesis 20 presumably normal rabbits were killed and the described tissues from both eyes of each taken for water determination. On the basis of differences in per cent water content between paired membranes, the mean difference and its standard deviation was found to be 0.685 ± 0.534. While the agreement is not exact, in view of the magnitude of the water gain obtainable through cigarette smoke irritation, it does not invalidate the proposed method.

A further analysis of the data obtained above was made on the basis of the mean per cent water content of all the left eye membranes in the series and of all the right eye membranes, for if the deviations from the mean on such a basis should prove no greater than that between the two membranes of the same animal it would permit assignment of a standard moisture content to the unirritated tissue. The left membrane mean proved to be 82.59 ± 1.24 per cent and that of the right 82.71 ± 1.02 per cent. Since these standard deviations from the mean are greater than those found between paired membranes, use of a standard water content for unirritated tissue was not adopted.

(b) *Effect of manipulation on the membrane water content.* To evaluate the irritant action of the cigarette smoke *per se*, it is necessary to know the effect on the tissue of the manipulations involved in applying the smoke. To this end 14 rabbits were subjected to Procedure A, with the single exception that 3 puffs of air instead of smoke were drawn past the right eyes. The results showed that the membranes of right eyes contained a mean of 76.87 ± 1.54 per cent water and those of left eyes 77.92 ± 1.54 per cent. We can therefore conclude that any edema produced by cigarette smoke in this method represents a true effect of the smoke.

(c) *Effect of graded exposure to cigarette smoke irritation on edema development.* To study this, 6 groups of 10 animals each were subjected to 1, 2, 3, 4, 5 or 6 puffs of smoke using Procedure A. Calculation of regression of the resulting moisture to dry weight ratios showed a highly significant regression coefficient (P equals less than .002). It is therefore concluded that the upper palpebral membrane responds to graded exposure to cigarette smoke irritation by graded increases in edema formation.

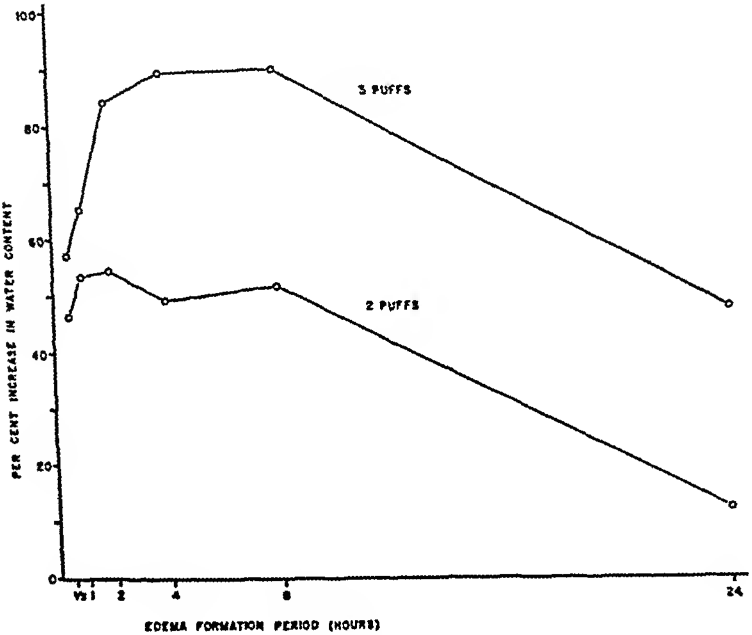

FIG. 2

It was also observed in these experiments, that at the higher puff levels there was an increasing likelihood for error in edema determination owing to seepage of fluid from the cut surfaces of the membrane during excision.

(d) *Selection of the number of puffs of smoke and the duration of edema development to use in making comparisons.* To determine this, cigarettes from one lot of tobacco were selected and rabbits exposed to 2 and 3 puffs of smoke using Procedure A. The rabbits were then killed at varying time intervals following exposure and the described tissues removed for water determination. The results are shown in figure 2 in which each point represents a mean of results on 20 animals.

From this it is seen that maximum edema development following exposure to 2 puffs of cigarette smoke occurs at the end of one hour, whereas, following

exposure to 3 puffs maxinum edema is reached only after four hours. However, some of the individual edemas produced in four hours at the 3 puff level are so great that considerable fluid exudes and is lost from the cut surfaces in excising the tissue. The degree of edema developed by 3 puffs in one hour is about as great as can safely be used without introducing errors due to such exudation. Therefore, whether 2 or 3 puff exposures are used, a one hour period for edema development appears optimum, and this has been used in all of the work to follow.

Since one puff exposures produce relatively little edema and 4 or more puffs may produce too much edema, the choice of the number of puffs to use would appear to be either 2 or 3. To test the relative efficiency of these puff levels in making comparisons of irritation, the following experiment was performed. Cigarettes of two different brands (a "king" size vs an ordinary size) were compared at both the 2 and 3 puff levels using Procedure B. Two groups of 10 comparisons were made at each puff level, and the results analyzed statistically. At the completion of the first 10 comparisons analysis of variance of the resulting moisture to dry weight ratios gave a P value for significance in difference in irritating properties between the two brands of about .05 at the 2 puff level and less than .01 at the 3 puff level. The second 10 comparisons gave similar P values to those obtained in the first series. From this it would seem that ordinarily 3 puffs is the more satisfactory number to use in making comparisons of the irritating properties of cigarette smoke by the described method.

(e) *Physical standards for cigarettes.* Despite the mechanical precision with which modern cigarettes are made, they tend to vary in the tightness of the packing of the tobacco. This is reflected in variations in weight as well as in resistance to air flow from one cigarette to another. When such variations between cigarettes are large differences in the irritant properties of their smoke due to variations in degree of combustion of the tobacco, concentration of the smoke, etc., may occur. We have not controlled this factor in testing different brands, the cigarettes in such cases being selected for comparison one with the other on the basis of similar package position. However, in studying experimental cigarettes in which but a single change has been made from the control cigarette (i.e. addition or omission of a single ingredient), weight and air flow variations become an undesirable complication. In such cases we have, from a predetermined knowledge of the average weight of the cigarette used, selected for smoking purposes only those that weighed within ±5 per cent of the average. In practice, it was found that restriction of the weight range automatically, with but few exceptions, restricted the range for air flow values. Within the above weight range, we have not been able to correlate differences in irritant properties between individual cigarettes of the same type with differences in their weights.

To illustrate the importance of standardization of the weight range of cigarettes, 20 cigarettes of the same brand were selected that represented extremes in weight range. Ten of these ranged between 920 and 960 mg. in weight (mean 939 mg.); the 10 at the other extreme ranged between 1200 and 1285 mg. in

weight (mean 1218 mg.). Rabbits were subjected to 3 puffs of smoke from these cigarettes using Procedure A. Water increases in the upper lid membranes of the exposed eyes gave a mean value of 78.98 per cent for the 10 light cigarettes and 39.53 per cent for the 10 heavy cigarettes. Analysis of variance of the corresponding moisture to dry weight ratios indicated a probability of less than .01 that this difference was due to chance.

Another factor which definitely affects the irritant properties of cigarette smoke is the moisture content of the tobacco. In the results reported here, this has been controlled by keeping the cigarettes to be smoked in a constant humidity cabinet for at last 24 hours prior to smoking and by moisture assays at the time of smoking.

To demonstrate the importance of controlling the moisture content, cigarettes selected as to weight were subjected to atmospheres of differing humidity to produce differences in their moisture content. Rabbits were then subjected to 3 puffs of smoke from these cigarettes using Procedure A. The results are presented in table 1.

TABLE 1

MOISTURE CONTENT OF CIGARETTES	NO. OF ANIMALS EXPOSED	MEAN INCREASE IN MEMBRANE WATER CONTENT
per cent		*per cent*
8.67	10	116.4
10.36	10	92.3
12.33	20	90.0
13.99	10	68.9
16.19	10	55.3
22.95	10	17.1

Analysis of variance of the corresponding moisture to dry weight ratios indicated a probability of less than .01 that these differences were due to chance.

SOME RESULTS OF APPLICATION OF THE METHOD. 1. *Comparison of irritating properties of smoke from cigarettes of the same brand standardized as to physical properties.* In the foregoing, we have described a method for evaluating quantitatively the irritating properties of cigarette smoke as judged by degree of edema formation and have shown that certain physical properties of cigarettes are possible influencing factors. The question remains as to whether or not cigarettes can be manufactured with sufficient uniformity as to constituents so that demonstrable differences in irritating properties do not occur between cigarettes of the same brand despite uniformity in physical properties. To test this, 100 cigarettes of one brand that did not vary from the mean weight by more than ±5 per cent were selected and divided at random into two groups of 50. The cigarettes were then brought to uniform moisture content and members of one group paired at random with those of the other and compared by Procedure B. Analysis of variance for difference in irritation between the two groups indicated that the probability of a more divergent sample would be about .23 (one animal

was eliminated from the analysis because of an exceedingly abnormal degree of edema formation). Thus, no significant difference in irritating properties was detected between the two groups.

2. *Comparison of irritating properties of smoke from cigarettes of two different brands.* We have seen that cigarettes of the same brand can be of sufficiently uniform composition so as to present no detectable significant difference in the irritating properties of their smoke. Of even greater interest in the application of the described method is whether or not significant differences in irritating properties may exist between smoke from cigarettes of different brands. To determine this 50 cigarettes from each of two different brands were compared by Procedure B. These cigarettes were all brought to comparable moisture content (12.07 vs 11.72 per cent) prior to testing, but were not standardized as to weight, since in the testing of different brands this did not seem to us to be a factor over which we should exert control. The 50 cigarettes representing each brand were selected on the basis of similar package position and pairings for testing were made on the same basis. Statistical treatment of the results gave a P value of .02 indicating that a significant difference existed. Tightness of packing of the tobacco seemingly did not contribute to this difference since the mean weight of the cigarettes of one brand was 1.084 $\pm$ 0.078 gm. and that of the other 1.096 $\pm$ 0.073. A subsequent check on mean circumference and length showed no significant differences. Thus it is seen that measurable differences in irritating properties may exist between different brands of cigarettes.

3. *The role of hygroscopic agents in cigarette smoke irritation.* This question has evoked considerable debate in recent years, duc largely, we believe, to the fact that results obtained by previously proposed methods for evaluating cigarette smoke irritation have depended in most cases on visual estimation, the vagaries of which are well known. We have therefore undertaken to study this question by our method. Since the two hygroscopic agents most commonly used in cigarette manufacture are glycerine and diethylene glycol we have submitted these to the following studies.

One lot of tobacco, of as near uniform composition as can technically be obtained, was divided into three lots. One lot was treated with glycerine in amount equal to 3.65 per cent by weight of the tobacco used. One lot was treated with diethylene glycol in amount equal to 2.74 per cent by weight of the tobacco used. The third lot served as a control and accordingly was not treated with a hygroscopic agent. Each of the lots was then made into cigarettes from which an adequate number were selected as to weight and brought to moisture content comparable to that normally found in market cigarettes by exposure to an atmosphere of constant humidity (65 per cent relative humidity at 25°C.) The moisture values attained were: control cigarettes 11.32 per cent, glycerine treated cigarettes 12.72 per cent, and diethylene glycol treated ones 12.36 per cent. From table I it may be seen that moisture content difference of these magnitudes do not significantly alter the edcma-producing properties of cigarette smoke. Fifty such cigarettes from each lot were tested by Procedure A and in addition 50 from each of the two lots containing hygroscopic agents were tested by Pro-

cedure B. For purposes of blind testing, the identity of each lot was concealed by code.

Analysis of variance of the results obtained by Procedure A yielded a P value of about .50, indicating no significant difference in irritating properties among the three lots. Statistical analysis by the same method of the results obtained by Procedure B gave a P value of about .30 thus confirming the results obtained by Procedure A. It would, therefore, seem that neither of these hygroscopic agents contribute significantly *per se* toward altering the irritating properties of cigarette smoke.

4. *Comparison of the irritant properties of smoke solutions with those of whole smoke.* In the introduction it was stated that the irritant properties of whole smoke differ markedly from those of smoke solutions. This difference is illustrated by the following experiment:

A smoke solution was prepared by allowing the first 3 puffs of smoke from each of 44 cigarettes to settle by gravity onto the surface of 4 ml. (88 drops from the medicine dropper selected for use) of 0.85 per cent sodium chloride solution. After thorough agitation the solution was filtered and 2 drops (equivalent to the soluble ingredients in 3 puffs of smoke) were instilled into the right eye of each of 10 rabbits and held in place for 3 minutes. Two drops of 0.85 per cent sodium chloride solution were instilled into each left eye. Following a period of one hour for edema development, the described tissues were excised and their water contents determined. The tissues exposed to the smoke solution contained a mean value of 1.22 ± 3.20 per cent more water than did those exposed to saline solution. Thus, tissues exposed to the soluble ingredients of 3 puffs of cigarette smoke showed no significant gain in water content as compared to a mean gain of 65.4 per cent (see figure 2) when exposed to 3 puffs of whole smoke.

SUMMARY

A quantitative method for evaluating the edema producing properties of the irritants in cigarette smoke has been described. Application of the method has demonstrated the following:

1. Tightness of packing of the tobacco within a cigarette can significantly alter the irritant properties of its smoke. The tighter the packing the less irritating is the smoke.

2. The irritant properties of cigarette smoke vary inversely with the moisture content of the tobacco smoked.

3. Cigarettes of the same brand may be of sufficiently uniform composition as to constituents so that no significant difference in irritant properties of their smoke can be detected.

4. The smoke from different brands of cigarettes may differ significantly in irritant properties.

5. The hygroscopic agents, glycerine and diethylene glycol, do not *per se* significantly alter the irritant properties of cigarette smoke.

6. The irritant properties of cigarette smoke directly applied are markedly greater than those of comparable cigarette smoke solutions.

While the described method has been here directed toward measurement of cigarette smoke irritation, we have found that by modifying the mode of administration of the irritant to suit the need, the method is equally applicable to the measurement of the edema-producing properties of gases, vapors, and liquids in general.

Acknowledgment. We are indebted to Professor George W. Snedecor, Director of the Statistical Laboratory of Iowa State College, for advice and aid in the statistical analyses used.

BIBLIOGRAPHY

1. Mulinos, M. G., and Osborne, R. L.: Proc. Soc. Exper. Biol. & Med. 32: 241, 1934.
2. Weatherby, J. H.: J. Lab. & Clin. Med. 25, 1199, 1939–40.
3. Flinn, F. O.: Laryngoscope 45, 149, 1935.
4. Ballenger, H. C. and Johnson, V. H.: Arch. Otolaryng. 25: 75, 1937.
5. Ballenger, H. C.: Arch. Otolaryng. 29: 115, 1939.
6. Proetz, A.: Ann. Otol., Rhin. & Laryng. 48, 176, 1939.
7. Holck, H. C. O., and Carlson, A. J.: Proc. Soc. Exper. Biol. & Med. 36: 302, 1937.
8. Bradford, J., Harlan, W., and Hanmer, H. R.: Indust. & Engin. Chem. 28, 836, 1936.

THE CHRONIC TOXICITY AND PATHOLOGY OF THIOURACIL IN CATS

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Received for publication, October 30, 1946

In the course of investigations on the pharmacologic action of alphanaphthylthiourea (ANTU) it was observed (1) that continued feeding of the substance to cats in daily doses of 10 to 20 mg. per kg. for about three weeks resulted in a rapidly progressive bilirubinemia with fatal termination in a few days. Histopathologic studies revealed fatty degeneration of the liver and intrahepatic obstruction, with bile casts in the bile capillaries and proliferation of small bile ducts. The similarity of this substance to thiouracil in chemical structure and the reported occurrence of jaundice in some patients treated with thiouracil (2) prompted this investigation.

EXPERIMENTAL. Twelve full grown cats weighing 2.2 to 3.2 kg. kept in individual cages were divided into four groups, three each, and fed daily doses of 100, 200, 300 and 500 mg. per kg. of thiouracil respectively incorporated into the daily ration of about 100 grams of lean ground beef. The animals were weighed once weekly. At frequent intervals blood examinations were made for hemoglobin, leucocyte counts, for abnormalities in stained Wright's smears, and for plasma bilirubin. The feeding experiment was continued for about 7 to 8 months. At the end of the experimental period liver function tests were made on the survivors by determining the rate of disappearance of a standard dose of intravenously injected bilirubin, by the method previously described (10). The animals were then killed with chloroform and histologic examination made of the liver, spleen, kidney, thyroid, testis, suprarenal, and pituitary.

RESULTS. *General symptoms and mortality.* No abnormal effects were noted throughout the experimental feeding period in the nine animals receiving up to 300 mg. per kg. per day. They ate their food well, and maintained their body weight, or made what may be considered normal gains. Only one animal in this series of nine died after 2½ months. At autopsy there was a small amount of pericardial effusion and one kidney was much enlarged. Microscopic examination did not reveal anything abnormal but suggested pleural and peritoneal exudate. It is not likely that this had anything to do with the thiouracil. All the other eight animals survived the full experimental period, and appeared to be in good condition when the experiment was terminated. The three animals receiving 500 mg. per kg. died in 38 to 90 days.

Hematologic studies. Leucocyte counts were made at 2, 3 and 6 months after the start of the experiment. These varied from 7000 to 20,000 per cu. mm. The usual range was 12,000 to 15,000 with 50 to 80% polymorphonuclears, 5 to 10% eosinophiles and 10 to 40% lymphocytes. Blood smears failed to reveal anything abnormal except for the presence of moderate degrees of anisocytosis and a few Howell-Jolly bodies, which is not abnormal for cats. An occasional normo-

blast was found in two of the animals. Hematocrit studies made three months after the start of the experiment showed a range of 21 to 30% with an average of 25.4, and 1½ months later it was 18 to 26% with an average of 22.1%. In a series of seven normal cats the range was 22 to 29% with an average of 25.7%.

Examination of the plasma for bilirubin from time to time failed to reveal anything beyond traces, approximately 0.1 mg. %, and in most cases it was not detectable at all.

Hemoglobin determinations, in the cats receiving up to 300 mg. per kg. thiouracil daily, at the beginning and the end of the experiment were as shown in table 1.

TABLE 1

CAT NO.	DAILY DOSE	Hb (GM. PER 100)	
		Initial	Final
	mg. per kg.		
2	100	13.5	12.5
3	200	18.0	11.5
5	300	12.8	13.2
6	100	12.6	10.0
7	300	12.0	11.0
8	200	13.9	12.0
9	100	18.5	16.5
10	200	15.8	11.0
11	300	13.7	10.7

TABLE 2

CAT NO.	DAILY DOSE	2 HRS.	4 HRS.
	mg. per kg.	*mg. %*	*mg. %*
3	200	2.6	0.7
5	300	0.7	
6	100	0.7	0.5
7	300	0.9	0.5
8	200	0.8	0.3
9	100	1.0	0.2
11	300	0.7	0.5

These values are not greatly abnormal for cats, though there seems to be some indication of significant hemoglobin reductions in three of the animals, nos. 3, 10 and 11.

Liver function tests. The rate of disappearance from the plasma of a standard dose of 5 mg. per kg. bilirubin injected intravenously was studied in seven of the animals at the termination of the feeding experiment. The plasma bilirubin concentration at 2 and 4 hours after the injection in this group of animals was as shown in table 2.

In normal cats we had previously found at two hours a range of 0.4 to 0.5 and

at 4 hours 0.1 to 0.3 with an average of 0.2 mg. %. Retention should therefore be regarded as somewhat abnormal in four of the animals, nos. 3, 6, 7 and 11 and this probably indicates some degree of liver dysfunction.

Pathology. Autopsies were made on 12 cats which had received thiouracil over periods varying from 38 to 250 days. Four cats died at 38, 69, 79, and 229 day's, 5 were killed after 90, 205, 214, 219, 221 and the last 3 at 250 days. Grossly, minor fatty changes of the liver were inconstantly observed, icterus was noted in 1 cat, ascites in another. The thyroid was often quite small and hard to find. Testicular atrophy was noted in several male cats. Multiple yellowish miliary abscesses were present in the spleen in 1 cat.

Microscopic examination included routinely the liver, lung, spleen and kidney, usually the adrenal, heart and thyroid, and intestine, stomach, muscle, pancreas, ovary, testis, hypophysis and larynx in a variable number of cats.

The usual azure eosinate and iron hematoxylin-van Gieson stains were employed on all tissues. Hematoxylin and oil red O were used on frozen sections of liver, kidney, adrenal and heart. Ferrocyanide reactions were done on spleen, liver and kidney. Bensley Mallory anilin blue stains were tried on hypophysis but were found no better than azure eosin stains. Bauer glycogen stains were done on the livers.

In the liver the most consistent finding was a moderate to fairly severe, fine and medium fat droplet infiltration of liver cells, occurring in irregular areas, sometimes patchy or focal, chiefly periportal in 3 cats, centrolobular in 4. Centrolobular congestion was noted in 3 cats, atrophy in 2 of them. Cytoplasmic oxyphilia of liver cells in the lobule centers accompanied the centrolobular fatty change in 2 cats. In 1 cat, dead in 69 days after 40 doses of 500 mg. per kg., there was a partial portal cirrhosis. Moderate to large amounts of glycogen were demonstrated in the livers of 6 cats killed at 90, 205, 214, 219, 221, and 250 days, even though most of these showed a considerable amount of fatty degeneration. In the 4 cats which died only traces or no glycogen remained. This may be purely an autolytic change, though glycogen may remain demonstrable for considerable periods post mortem. In 2 cats killed at 250 days, glycogen was absent and small in amount respectively.

Another prominent hepatic alteration was a slight to quite severe granular hemosiderin pigmentation of the hepatic littoral (Kupffer) cells. This appeared in 9 cats, and in 5 of them there was also a moderate hemosiderosis in the splenic pulp.

Otherwise the spleen presented no consistent alterations. A slight to moderate myelosis was noted in 4 cats, focal abscesses and fibrinopurulent perisplentitis in 1 of these 4, follicle hyperplasia in 3 cats, and a considerable number of mast cells in the pulp in 2.

The renal cortex presented the fairly heavy fatty infiltration of the epithelium of the convoluted tubules which appears to be normal in cats. The only pathologic finding was an exudative interstitial nephritis occurring in 4 of the 12 cats, and accompanied by pyelitis in 2 of them. The infiltrating cells were chiefly lymphocytes. In 1 case there were many plasma cells as well, and scattered

karyorrhectic and purulent capillary thrombi were present. This nephritis is probably to be regarded as intercurrent in nature.

The adrenal medulla ordinarily contained moderate to large amounts of chromaffin substance. Usually the fascicular zone of the cortex contained considerable amounts of fat and some bi-refringent lipoid, the glomerular and reticular zones less. In a cat which died 2 days after 1 gm./kg. dosage per day there was extensive necrosis, hemorrhage and calcification in the reticular zone of the adrenal cortices extending somewhat into the meduallа in 1 adrenal and into the fascicular zone in both. In 8 of the remaining 10 cats in which the adrenals were studied, there was slight to severe interstitial fibrosis, scarring and irregular atrophy in the reticular zone of the adrenal cortex, and in 1 of these (214 days), some necrotic cells were seen.

Heart muscle was essentially normal in 2 cats, slight to moderate, irregular or diffuse deposition of fine fat droplets in the muscle fibers was noted in 6 cats, and in 1 there was epinuclear lipochrome pigmentation. Heart muscle was not saved in 3 cats.

Skeletal muscle presented no significant lesions in the 4 cats in which sections were made.

Quite active mucus secretion by surface goblet cells and mucosal glands was noted in the intestine, particularly ileum and cecum, in all of the 4 cats in which these tissues were studied. The pancreas was normal in the 3 cats, in which it was studied. Stomach was noted as normal in all of 4 cats, esophagus in 2, various lymph nodes in 4.

Ovaries were noted as normal, with maturing ova and corpora lutea, in 2 cats. In both of these the tubal mucosa appeared rather fibrous and somewhat atrophic.

Sections of testis were made in 3 cats. The tubules of the epididymis were empty. The germinal epithelium of the seminiferous tubules was atrophic, foamy or vacuolated and reduced in thickness and spermatozoa were absent. Conspicuous masses of coherent, foamy polygonal interstitial cells were present in all 3.

Thyroid was sectioned in 9 cats. Acini were generally quite small, with tiny empty lumina and cuboidal epithelium. In most of the cats there were some larger acini, empty, or containing faintly to moderately oxyphil colloid, with or without peripheral vacuolation.

Only 2 parathyroids were found in the thyroid sections. These were quite small and histologically normal.

Hypophyses were sectioned from 4 cats. In each there were areas near the convex ventral surface of the anterior lobe which were composed mostly of large oxyphil cells. The more central portion of the anterior lobe contained a more nearly equal mixture of oxyphil cells, chromophobe cells and lightly basophil cells. In the pars intermedia the two latter cell types predominated.

Discussion. Considering the doses given and the length of time the animals were exposed to the drug the effects were relatively slight. Since this work was completed a report has appeared by Aranow and associates (3) on the effects of feeding thiouracil up to 0.8 gm. per day to adult rhesus monkeys for 14 months.

No important toxic effects were observed except for a neutropenia which developed in two of their animals.

The adrenal cortical atrophy observed in the present series of experiments ties in well with other observations. Glock (4) reported suprarenal cortical deficiency with depletion of lipoids in rats fed thiourea or thiouracil. This investigator also reported depletion of liver glycogen in chronic thiouracil poisoning in rats. Six of the 8 cats killed and immediately autopsied retained very considerable amounts of liver glycogen in our series. Raab (5) observed a decreased sensitivity of the heart to epinephrine in normal human subjects receiving thiouracil for 3 months. Marine and Baumann (6) state that the suprarenals in rats chronically poisoned with thiouracil presented hypertrophy of the adrenal medulla, the cells taking on a more intense chrome stain than normal, and suggest that there may have been an increase in the epinephrine content, since they found an increased amount of chromogenic substances as measured by the Folin-Cannon method. Essentially our histologic findings on the cat adrenals agree. It should be of great interest to ascertain the physiological vasopressor response in thiouracil poisoned cats to minimal doses of epinephrine and other sympathomimetic drugs. Paschkis and associates (7) studied melanin formation in a tyrosinase-tyrosine system and observed that this was inhibited by thiouracil. Inhibition of tyrosinase should protect epinephrine from destruction, and this might very well explain the foregoing observations.

As regards the action of thiouracil on the hematopoietic system our clinical laboratory findings were essentially negative, although there is some evidence of blood destruction in the hepatic and splenic hemosiderosis. Apparently the diet the cats were receiving, ground lean beef, is so adequate for this species that it compensated for the injurious effects that the thiouracil might have had. Vogel and Gavack (8) administered thiouracil to rats in doses of 50 to 225 mg. daily for a period up to 78 days and only found a slight decrease in the red cells and hemoglobin and no significant change in the leucocyte count, though bone marrow smears revealed a decrease in the myeloid-erythroid ratio. The administration of thiouracil in the presence of dietary deficiencies would almost certainly produce an entirely different picture.

Finally the observations of Gyorgy and Goldblatt (9) should not pass unmentioned. These observers found that 0.1% thiouracil in a cirrhosis producing diet in rats protected against liver injury. It is their opinion that by inhibiting thyroxine formation thiouracil lowers the metabolic rate and thus has a saving effect on the proteins and the amino acid methionine so essential in the prevention of liver cirrhosis. It may well be that the thiouracil given to our cats subsisting on a high protein diet had the effect of affording protection to the liver which might have otherwise suffered injury if the thiouracil had been given in a less favorable diet.

SUMMARY

Thiouracil fed to cats in doses of up to 300 mg. per kg. per day for a period of 7 to 8 months had no demonstrable toxic effects, it had no appreciable effect on

the hematocrit, it had an inconstant effect on the hemoglobin, no effect on plasma bilirubin, and only slight effect in delaying the rate of elimination of intravenously injected bilirubin in about half of the animals. Daily doses of 500 mg. per kg. in three cats proved fatal in 38 to 90 days.

The significant pathologic findings appear to be necrosis and scarring in the reticular zone of the adrenal cortex, moderate fatty degeneration in the liver, with glycogen preservation, atrophy of the thyroid and of the seminiferous tubules of the testis, hyperplasia of testicular interstitial cells and a moderate hepatic and splenic hemosiderosis.

REFERENCES

1. McClosky, W. T., Smith, M. I., and Lillie, R. D.: Pub. Health Rep., 60: 1101, 1945.
2. Cargill, S. L., and Lesses, M. F.: Jour. Am. Med. Assoc., 127: 890, 1945.
3. Aranow, H., Engle, E. T., and Sperry, W. M.: Endocrinology, 38: 331, 1946.
4. Glock, G. E.: Nature, 156: 508, 1945.
5. Raab, W.: Jour. Lab. and Clin. Med., 30: 774, 1945.
6. Marine, D., and Baumann, E. J.: Am. J. Physiol., 144: 69, 1945.
7. Paschkis, K. E., Cantarow, A., Hart, W. M., and Rakoff. A. E.: Proc. Soc. Exper. Biol. & Med., 57: 37, 1944.
8. Vogel, M., and McGavack, T. H.: This Journal, 86: 120, 1946.
9. Gyorgy, P., and Goldblatt, H.: Science, 102: 451, 1945.
10. Smith, M. I., Westfall, B. B., and Stohlman, E. F.: Nat. Inst. Health Bulletin No. 174, 23, 1940.

STUDIES ON THE PHARMACOLOGY OF BETA-DIETHYLAMINOETHYLDIPHENYLTHIOACETATE, A SYNTHETIC ANTISPASMODIC

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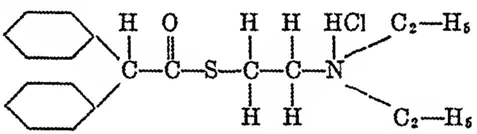
Received for publication October 30, 1946

In recent years many new synthetic compounds with varying degrees of antispasmodic activity have been introduced. A comprehensive survey of those described before 1943 has been published by Raymond (1). Nearly all of these compounds have been esters, and invariably these esters have been carboxylic acid compounds. The compound presented here, beta-diethylaminoethyldiphenylthioacetate,[1] is unusual in that it is the ester of a thio-acid; it may indicate the potentialities in this field of a whole new group of compounds. It bears at present no commercial name and will be referred to by its serial number, 23B.

CHEMISTRY. The compound 23B is the salt of a basic ester of a thio-acid, with the structural formula:



It is a white crystalline compound, very soluble in alcohol and water, slightly soluble in acetone and ethyl acetate, and very slightly soluble in ether and the other common organic solvents. It is not recommended that solutions of 23B be boiled since such treatment causes it to be broken down into the components of the ester. The pH of a 1.0 per cent solution is 5.2. Upon ignition the compound is completely consumed leaving no ash.

PHARMACOLOGY. *Toxicity.* The toxicity of the compound was determined by establishing the LD50 for its administration by intravenous, intraperitoneal and oral routes. The animals used in these determinations were male mice, rats rabbits and dogs; food was withheld for 16 hours before use. In many instances identical tests were carried out using the drug Trasentine, Ciba, (diphenylacetyldiethylaminoethanol), which has chemical characteristics and pharmacological effects similar to those of 23B (1, 2, 3). The Trasentine employed was that marketed in ampuls containing ammonium chloride. The data for these toxicity studies are tabulated in Table I. The method of Behrens (4) was used to calculate deduced mortality.

The findings on intravenous toxicity of 23B to dogs were substantiated by the fact that when anesthetized dogs used for other purposes were killed at the end

[1] Supplied by the research laboratories of Wm. P. Poythress and Company, Inc., Richmond, Virginia.

TABLE I

Toxicity of the compound 23B and of Trasentine

ANIMAL	ROUTE OF ADMINISTRATION	DRUG	DOSE	NUMBER OF ANIMALS	NUMBER DEAD	DEDUCED MORTALITY	LD50
			mgm./kg.			%	mgm./kg.
Mouse	Intravenous	23B (0.1%)	10	20	2	3.9	30.5
			20	20	3	13.5	
			30	20	9	48.3	
			40	20	16	88.2	
		Trasentine (0.1%)	10	20	0	0.0	28.7
			20	20	3	9.4	
			30	20	12	55.5	
			40	20	16	88.6	
Rabbit	Intravenous (each injection in 40 sec.)	23B (1%)	15	10	0	0	19.1
			20	10	6	60	
			25	10	10	100	
Dog	Intravenous (each injection in 60 sec.)	23B (5%)	10	2	0	0	30
			20	2	1	50	
			30	2	1	50	
			40	2	2	100	
		Trasentine (3.3%)	20	2	0	0	35
			30	2	0	0	
			40	2	2	100	
Mouse	Intraperitoneal	23B (1%)	100	20	1	3.4	187
			200	20	11	57.1	
			300	20	20	100	
		Trasentine (1%)	100	20	1	2.6	229
			200	20	6	31.8	
			300	20	19	96.3	
Rat	Intraperitoneal	23B (5%)	1000	1	0	0	1500
			1200	3	1	15	
			1500	5	1	33	
			2000	3	3	100	
Mouse	Oral	23B (total vol. = 30 cc./kg.)	300	20	4	11.8	443
			450	20	11	51.7	
			600	20	16	86.1	
			1200	20	20	98.1	
			1500	20	19	98.6	
		23B (total vol. = 30 cc./kg.)	300	10	1	4.6	700
			600	10	7	40.0	
			750	10	3	55.0	
			900	10	8	90.5	

TABLE I—*Continued*

ANIMAL	ROUTE OF ADMINISTRATION	DRUG	DOSE	NUMBER OF ANIMALS	NUMBER DEAD	DEDUCED MORTALITY	LD50
			mgm./kg.			%	*mgm./kg.*
Mouse	Oral	Trasentine (total vol. = 30 cc./kg.)	300	20	0	0	780
			600	20	7	17.9	
			750	20	6	40.6	
			900	20	15	84.8	
Rat	Oral	23B (10% in acacia)	2000	10	0	0	2720
			3000	10	7	70	
			4000	10	10	100	
Dog	Oral	23B (5% in acacia)	750	1	0	0	1500
			1500	2	1	50	
			2000	1	1	100	

of the experiment by intravenous injection of 23B, a comparable dose was found to be fatal.

Animals from the same lot were used for determination of toxicities of the two drugs; one exception to this plan was the set on oral toxicity of 23B to mice in which the LD50 was 443 mgm./kg. This was repeated with animals of the lot used for Trasentine and was found to check more properly with figures for that drug.

In some cases included in the table (notably intraperitoneal injection into rats and oral administration to dogs) the number of animals was insufficient for arrival at a true LD50; the figures listed in these instances represent approximations only.

Action of lethal and sub-lethal doses: Animals receiving fatal doses of the compound 23B died in convulsions of clonic character. Dogs and mice appeard to be more so affected than rats and rabbits. Sub-lethal doses produced nystagmus, loss of equilibrium, muscular weakness and incoordination, the hind quarters being affected first. Vomiting was produced by large oral doses (750 mgm./kg. and above) presumably through gastric irritation, because it rarely occurred when the drug was administered parenterally. Dogs into which 23B was injected intravenously died in about 2 minutes or were wholly recovered in 40 minutes. Dogs given the drug in large oral doses either died in 30 to 60 minutes or recovered fully in 90 minutes. Thus the toxic action of 23B, even in nearly fatal doses, appeared to be fleeting.

Effect on blood pressure. Intravenous injection of the compound 23B in doses equal to or greater than 0.5 mgm./kg. in the dog, anesthetized with sodium pentobarbital, caused a transient fall of blood pressure as indicated below (25 experiments). This effect was quantitatively similar to that produced by corresponding doses of Trasentine in the amounts tested (1 mgm./kg. and less).

When a fatal dose of 23B was injected intravenously there followed respiratory halt as well as an immediate fall of the blood pressure to zero. The heart continued to beat during this fall.

DOSE OF 23B	EFFECT
mgm./kg.	
0.1	No effect
0.5	In general a fall in blood pressure of 2 to 10 mm. Hg, of 10 to 15 seconds' duration, occasionally no effect or a negligible rise in pressure.
1.0	A fall of 2 to 10 mm. Hg of 10 to 15 seconds' duration
5.0	A fall of 50 to 60 mm. Hg of about 2 minutes' duration
10	A fall of 60 to 80 mm. Hg of about 2 minutes' duration
20	A fall of 100 mm. Hg of 3 to 4 minutes' duration

Effect on uterine muscle. In order to investigate the antispasmodic action of the compound 23B upon uterine muscle, experiments were arranged to compare its inhibitory action upon uterine contractions with that of several other antispasmodic drugs, particularly with the drug Trasentine, both in excised tissue and in the intact animal.

TABLE II

Action of antispasmodic drugs on uterine muscle

DRUG	EFFECTIVE CONCENTRATION		
	Vs normal contractions (rat uterus)	Vs $BaCl_2$ 1:2000 (rat uterus)	Vs $BaCl_2$ 1:10,000 (human uterus)
Papaverine hydrochloride	1:133,300	1:131,300	1:25,000
23B	1:78,700	1:80,800	1:25,000
Trasentine	1:42,200	1:46,500	1:14,300
Pavatrine	1:16,600	1:18,800	
Syntropan	1:4,400	1:3,600	

In vitro: In these studies isolated uterine segments from 5 month old virgin rats were suspended in oxygenated Locke-Ringer's solution maintained at 37°C.; the drugs to be tested were added to the muscle bath. Uterine contraction was recorded on a kymograph.

Thirty-six such preparations were studied to demonstrate the relative efficiency of five antispasmodic drugs in reducing uterine contractions. Drugs were tested against normal contractions and against spasm created by barium chloride in the concentration of 1:2,000. Results are tabulated in Table II. Figures listed are the minimal concentrations necessary to reduce the extent of contraction to one half its former value.

In two cases, segments of human uterus[2] were obtained for further evaluation of the drug. These sections were taken from the fundus of the uterus and consisted of longitudinal strips 30 mm. by 5 mm. Drugs were tested against spasm induced by barium chloride 1:10,000. The concentrations of drugs necessary to relax such spasm are also included in the table.

In vivo: Contraction of uterine muscle in situ was recorded in the dog and rabbit by means of the Jackson enterograph, and in the rat by means of Bar-

[2] Courtesy of Dr. William Bickers.

bour's technic (5). The latter animal and method proved to be the most satisfactory for our purposes and were used for the major part of the work. The arrangement consisted essentially of a single intact uterine horn attached by its distal end to a writing lever. The muscle was drawn up through an abdominal incision into which was fitted a glass cylinder filled with light mineral oil, which thus acted as a bath for the uterine horn.

With this arrangement 50 experiments were conducted in which the effects of subcutaneous and intravenous doses of 23B and Trasentine were recorded.

Normal contractions of the rat uterus could be inhibited by subcutaneous administration of either drug; 5 to 15 minutes after administration of 100 mgm./kg. of 23B complete inhibition was produced which lasted more than 250 minutes (fig. 1). Trasentine in the same dose had a similar but slightly less marked inhibitory effect.

Injection of 75 mgm./kg. produced temporary inhibition or a partial effect from which the muscle was able to escape from time to time. Doses of 50 mgm./kg. of

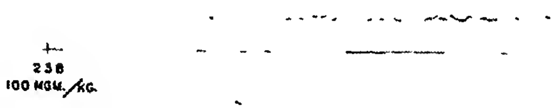


FIG. 1. ACTION OF SUBCUTANEOUS INJECTION OF 23B UPON NORMAL CONTRACTION OF RAT UTERUS

both drugs were mildly effective, decreasing tone and amplitude slightly. Doses of 30 mgm./kg. administered subcutaneously had practically no effect in either case.

When injected intravenously the drugs were effective in smaller doses. Injection of 10 mgm./kg. of 23B markedly decreased the amplitude of normal uterine contractions, not quite abolishing them altogether; this effect persisted for about 120 minutes. Trasentine given in the same dose had a somewhat less marked effect, reducing contractions to about one half of their former height; the duration of the effect was about the same as for 23B.

In addition to testing the drugs against normal uterine contractions, their antispasmodic effect was compared by testing them against spasm induced by subcutaneous injection of 2 cc./kg. of Pitocin, N.N.R. This induced vigorous contractions which were maintained for more than two hours. Both 23B and Trasentine in doses of 150 mgm./kg. given subcutaneously completely inhibited these contractions; from 5 to 40 minutes were required for the onset of the 23B effect and about 40 minutes for that of Trasentine. The duration of the effect was very long for both drugs, around 200 minutes.

In doses of 100 mgm./kg. 23B decreased the amplitude to a state of minimal

contraction in 40 to 100 minutes (fig. 2). Inhibition was somewhat less than that produced by 23B. The effect of both drugs was maintained for hours.

A dose of 75 mgm./kg. had a mildly inhibitory action under these conditions, for both 23B and Trasentine.

Results obtained with the dog and rabbit as experimental animals were consistent with the above.

Effect on the gastro-intestinal tract. A series of studies was made on the action of the compound 23B and that of Trasentine upon contraction of intestinal muscle; these were carried out in several ways, preliminary tests performed in vitro, acute experiments upon anesthetized dogs, and experimentation with dogs bearing modified Thiry-Vella loops. In cases in which uterine and intestinal records were made simultaneously, it could be clearly seen that both drugs exerted a more powerful action upon intestinal movement than upon contraction of uterine muscle.

FIG 2 ACTION OF SUBCUTANEOUS INJECTION OF 23B UPON PITOCIN-STIMULATED CONTRACTION OF RAT UTERUS

In vitro. A few experiments were carried out in which sections of excised rabbit intestine were suspended in Locke-Ringer's solution and tested in the same manner as that used for excised uterine muscle. Spasm of the muscle was first induced by acetylcholine bromide in a concentration of 1:1,000,000. The minimal effective dose of each of four drugs was determined:

DRUG	EFFECTIVE CONCENTRATION
Papaverine hydrochloride	1:133,000
23B	1:200,000 to 1:400,000
Trasentine	1:80,000
Pavatrine	1:19,000

In vivo: In 28 experiments upon dogs anesthetized with sodium pentobarbital, the action of the compound on intestinal movement was investigated with the aid of the spasmogenic drugs, physostigmine and pilocarpine.

When pilocarpine hydrochloride was injected intravenously (0.1 mgm./kg.) an increase of tonus of the intestine resulted which could be abolished for 30 minutes by the intravenous injection of 1 mgm./kg. of 23B. Under the same circumstances 0.1 mgm./kg. of atropine sulfate was less effective but its action endured for a longer time.

Twice this dose of pilocarpine (0.2 mgm./kg.) was used as the spasmogenic drug in a comparative study of 23B and Trasentine. Equal doses of these two drugs produced equal responses in the same animal; however, there was much variation among the various dogs' reactions. The minimal effective dose of both drugs appeared to be about 0.5 mgm./kg.; this produced partial relaxation of the pilocarpine-stimulated muscle in most cases.

Heightened tonus was also induced by intravenous administration of physostigmine salicylate. When 0.1 mgm./kg. of this drug was injected the resulting increase of tonus could be reduced by 1 mgm./kg. of 23B; for about 100 minutes

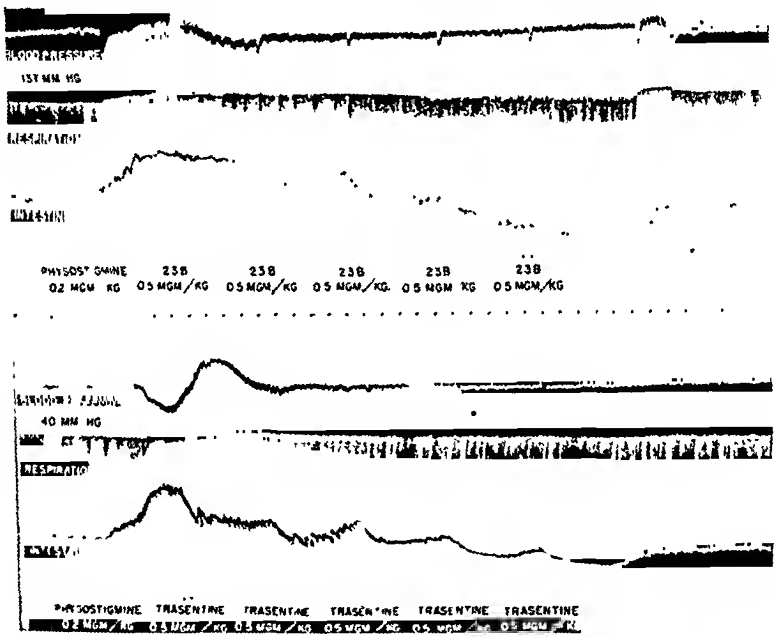


FIG. 3. EFFECT OF INTRAVENOUS INJECTION OF 23B AND OF TRASENTINE UPON BLOOD PRESSURE, RESPIRATION AND INTESTINAL TONE IN THE DOG

thereafter additional physostigmine was without effect on the intestine. With a larger dose of physostigmine (0.2 mgm./kg.) the administration of 1 mgm./kg. of 23B was followed by a prolonged fall in tonus level.

This last-mentioned dose of physostigmine salicylate (0.2 mgm./kg. given intravenously) was employed in a relatively accurate comparison of the antispasmodic effects of the two drugs 23B and Trasentine upon the gastro-intestinal tract of the anesthetized dog. Five minutes after the injection of physostigmine, one of the drugs to be tested was injected (0.5 mgm./kg.); this was repeated at 5 minute intervals until 5 injections of the drug had been made (fig. 3). Five such assays were conducted; both drugs were tested on each animal used, the order of

their administration being reversed in successive cases. An immediate drop in tonus resulted from each such injection producing a stepwise dissolution of the physostigmine stimulation.

The compound 23B showed a slight advantage over Trasentine in these assays. The third or fourth dose of 23B generally brought the state of tonus down to its original level, whereas Trasentine required 5 doses for this action, or, as in two cases, never accomplished it at all. Recovery from the action of the drugs was rapid, occurring in about 15 minutes in the case of 23B and in 5 to 10 minutes in the case of Trasentine. In many instances, with both drugs, the action of physostigmine was still obvious after the disappearance of the effects due to the antispasmodic drugs.

In dogs with Thiry-Vella loops: The compound 23B and Trasentine were used in a study on unanesthetized dogs in which subcutaneously placed Thiry-Vella

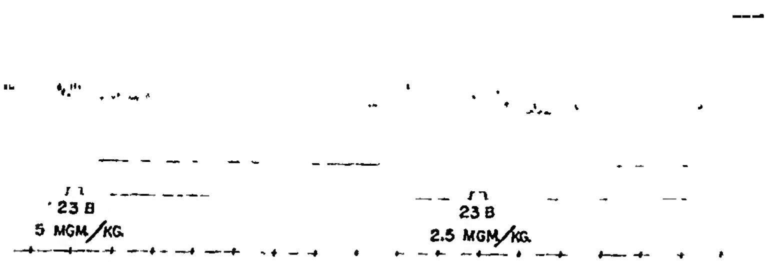


FIG. 4. EFFECT OF INTRAVENOUS INJECTION OF 23B UPON INTESTINAL TONE IN THIRY-VELLA LOOP OF THE UNANESTHETIZED DOG

loops of intestine had been constructed. The effect of 23B upon the normal contraction present in the loop and that of both drugs upon the increased contraction stimulated by the administration of physostigmine were investigated. An intravenous dose of 23B equal to 5 mgm./kg. was sufficient to reduce normal contraction to a minimum (fig. 4). When contraction of the intestinal segment was produced by intravenous administration of 0.2 mgm./kg. of physostigmine, this stimulation could be reduced or inhibited by intravenous injection of 5 mgm./kg. of 23B. The effect of this dose of 23B was consistently greater than that of a corresponding dose of Trasentine.

Prevention of diarrhea: Antispasmodic agents may be expected through their reduction of intestinal tone to counteract to a certain extent the effect of laxative drugs. Use was made of a technic introduced by Geiger (6) and improved by Hazelton and Fortunato (7) and by Grote and Woods (8) for studying diarrhea in mice in a series of four experiments; in these the ability of the compound 23B and Trasentine to check or prevent diarrhea induced by administration of senna was measured.

All mice were given by oral dosage 500 mgm./kg. of standard Tinnevelly senna

powder[3] in suspension. The per cent incidence of diarrhea resulting from this dose varied considerably among the various groups of mice studied. In the first experiment 50 mice were treated with senna alone; 50 were given senna to which was added 23B so that it was administered in doses of 10 mgm./kg.; 50 mice were given senna mixed with Trasentine in the same dosage. In the second experiment this procedure was repeated with a higher dosage of the antispasmodic drugs, 50 mgm./kg.

It was found that mixing the drugs with senna tended to inactivate them in some way (as tested upon the rat uterus in vitro); this change was apparently not due to any alteration of pH of the solutions. It was necessary therefore to prepare and administer the drugs separately. One hour after administration of senna one of the two antispasmodic agents (10 and 50 mgm./kg.), or a comparable volume of Ringer's solution for the controls, was given orally. The results of the four experiments are tabulated in Table III.

TABLE III

Effectiveness of 23B and Trasentine in checking diarrhea in mice

All mice received 500 mgm /kg. senna

DOSE	INCIDENCE OF DIARRHEA IN PER CENT OF CASES		
	Control	23B	Trasentine
10 mgm /kg. mixed with senna	70	54	56
50 mgm./kg. mixed with senna	61.3	50.7	60
10 mgm /kg. given separately	62	46	58
50 mgm /kg. given separately	82	50	68

When the drugs were given an hour after dosage with senna their effectiveness became apparent. The incidence of diarrhea in mice treated with 10 mgm./kg. of 23B was 74.2 per cent of the incidence of diarrhea among the control mice; that among mice given 50 mgm./kg. of 23B was 61.0 per cent of that among control mice. Mice treated with 10 mgm./kg. of Trasentine showed diarrhea with 93.5 per cent of the frequency of its appearance among control mice; when Trasentine was given in doses of 50 mgm./kg. the incidence of diarrhea was reduced to 82.0 per cent of the control value.

Among mice showing diarrhea, the severity was somewhat lessened in animals treated with the antispasmodic drugs as compared with those receiving only senna.

Antisialogogue effect. An undesirable side effect of many of the spasmolytic drugs is the atropine-like drying of secretions. A comparative study was made of the action of 23B, Trasentine, and atropine upon salivary flow in a series of experiments on anesthetized dogs. Wharton's duct was cannulated and connected with a Gibbs drop recorder (9) arranged to indicate salivary flow on a

[3] Obtained through the courtesy of I. W Grote and Maribelle Woods

kymograph. Simultaneous records of salivation, blood pressure and intestinal movement were made. Salivary flow was induced by the intravenous injection of a 0.1 per cent solution of pilocarpine in the amount of 0.2 mgm./kg.; this caused a rapid flow lasting about two hours. When the injection of this amount of pilocarpine was followed after three minutes by intravenous injection of 0.5 mgm./kg. of 23B the salivary flow decreased or was inhibited altogether, according to individual variation among the animals, and resumed normal flow after 15 to 30 minutes. In each dog the antisialogogue effect of both 23B and Trasentine was tested against pilocarpine, the order of their administration being alternated in successive experiments. In these experiments the antisialogogue action of the compound 23B was slightly greater than that of the same dose of Trasentine. The decrease in salivary flow produced by 0.5 mgm./kg. of 23B corresponded to that produced by 0.0025 to 0.005 mgm./kg. of atropine sulfate; thus it appears to have only 0.5 to 1 per cent of the antisialogogue effect of that drug.

Irritation. The compound 23B acts as a local irritant in concentrations of 0.5 per cent and above. Six rabbits were used in an experiment in which intradermal injections of 23B and Trasentine were made. The drugs were dissolved in Ringer's solution to each of the following concentrations: (a) 5 per cent (23B only), (b) 1 per cent, (c) 0.5 per cent, (d) 0.1 per cent and (e) 0.01 per cent. Control injections of Ringer's solution were also made. All injections of either drug in concentrations of 5, 1, and 0.5 per cent produced local necrosis and sloughing of decreasing severity in the order named; the areas healed in about two weeks; induration and scar tissue remained thereafter. Little or no hyperemia was observed. The two drugs caused equal reactions in the same animal in all cases. In three of the animals the reaction to both drugs in 1 per cent dilution was very mild and in two animals it was moderate. In no case was there a reaction from the injection of either drug in concentrations of 0.1 per cent or less.

Irritation produced by local application was further investigated by dropping various concentrations of the compound 23B into the eyes of twelve rabbits. The left eye of each rabbit was used as a control, being treated with Ringer's solution. The following dilutions of the drug were used: (a) 2 per cent, (b) 1 per cent, (c) 0.5 per cent, (d) 0.25 per cent and (e) 0.125 per cent. In each case, three drops of such a solution were allowed to remain in the eye for three minutes, after which it was washed out with Ringer's solution. The strongest solution (2 per cent) produced marked edema and inflammation lasting more than four hours. Application of the 1 per cent solution produced moderate edema of 2 to 25 hours' duration and moderate inflammation of 1 to 2 hours' duration. The 0.5 per cent solution caused mild edema and inflammation (absent in one case) lasting about 30 minutes. Solutions of 0.25 and 0.125 per cent strength did not irritate the eye. The compound did not produce corneal pitting in any of the concentrations used here.

Multiple intravenous injections of strong solutions (1 to 5 per cent) of 23B resulted in local sclerosis of the vein at the site of injection.

Local anesthetic effect. The compound 23B is an effective local anesthetic, as

demonstrated by the test to be described. A comparison of its anesthetic action was made with that of cocaine hydrochloride as a standard of reference. Twelve rabbits were used; the right eye of each was treated with Ringer's solution to serve as a control. 23B in the following series of concentrations was allowed to remain in the test eye for three minutes: (a) 2.0 per cent, (b) 1.0 per cent, (c) 0.5 per cent, (d) 0.25 per cent, and (e) 0.125 per cent. The corneal reflex of each eye was tested before application of the drug and at 5 minute intervals thereafter. After application of the 2.0 per cent solution of 23B the cornea remained thoroughly anesthetized for 75 minutes, and returned to its normal condition only after 210 minutes. Upon application of the 1.0 per cent solution complete corneal anesthesia persisted for 34 minutes, and recovery of normal reflexes occurred 102 minutes after removal of the drug. With the 0.5 per cent solution complete anesthesia lasted for 27 minutes and reflexes returned to normal after 88 minutes. The 0.25 per cent solution caused complete loss of reflexes for 11 minutes, and recovery of the normal condition after 34 minutes. The weakest solution used, 0.125 per cent 23B, produced only a partial loss of the corneal reflex, returning to the full response after 19 minutes.

Upon comparison of these results with those obtained in a similar series using cocaine hydrochloride as the anesthetic agent, it was shown that the standard anesthetic action of 1.0 per cent cocaine was duplicated by that of 0.125 per cent 23B.

It may be pointed out that this drug, when applied locally to the rabbit eye, is an effective local anesthetic over a range including non-irritant doses.

Effect upon pupillary size. The compound 23B, when instilled into the rabbit eye, had no observable effect upon pupillary size or upon the light reflex. Six rabbits were used as experimental animals; the right eye of each was untreated except for instillation of Ringer's solution and served as a control. Into the left eye of each was placed 3 drops of one of the following dilutions of 23B: (a) 2.0 per cent, (b) 1.0 per cent, (c) 0.5 per cent, (d) 0.25 per cent, and (e) 0.125 per cent. The drug was allowed to remain in the eye for 3 minutes and was then washed out with Ringer's solution. The diameter of the pupil was measured both before and at regular intervals after application of the drug, and the light reflex was tested in both control and experimental eyes throughout the experiments. In no case was there any apparent effect, even when the drug solution was strong enough to irritate the eye.

SUMMARY

1. The pharmacological effects of Beta-diethylaminoethyldiphenylthioacetate hydrochloride (23B) as an antispasmodic drug were investigated.

2. The toxicity of the compound as expressed in terms of LD50 was relatively low. The LD50 by intravenous administration was 30 mgm./kg. for the dog, 19.1 mgm./kg. for the rabbit, and 30.5 mgm./kg. for the mouse. The LD50 by intraperitoneal administration was 1500 mgm./kg. for the rat, and 187 mgm./kg. for the mouse. The LD50 by oral administration was approximately 1500 mgm./kg. for the dog, 2720 mgm./kg. for the rat and 700 mgm./kg. for the mouse.

3. Intravenous injection of the compound produced a transient drop in blood pressure proportional to the dose.

4. In the excised rat uterus, addition of the compound 23B to the muscle bath reduced the muscle tone and amplitude of contractions. A concentration 1:78700 or less reduced normal contractions to one-half their former amplitude. A concentration of 1:80800 reduced muscle spasm produced by barium chloride 1:2000 to one half its tonus level in 15 minutes.

5. In the intact rat, subcutaneous administration of 50–100 mgm./kg. of the drug reduced or inhibited uterine contraction. A dose of 75 to 100 mgm./kg. reduced or inhibited spasm produced by administration of Pitocin.

6. In the anesthetized dog the effect of the drug upon intestinal motility was tested. Intravenous doses of 0.5 to 5 mgm./kg. resulted in partial or complete inhibition of intestinal movement, both that normally present and that stimulated by physostigmine. The drug had more effect upon intestinal muscle than upon uterine muscle in the same animal.

7. Normal intestinal contraction in the Thiry-Vella loop of the unanesthetized dog could be inhibited by intravenous administration of 5 mgm./kg. of 23B. The same dose reduced the stimulated contraction induced by physostigmine.

8. The compound 23B was effective in checking diarrhea in mice. The incidence of diarrhea produced by 500 mgm./kg. of senna was reduced to 61 per cent of the incidence among control animals by administration of 50 mgm./kg. of 23B.

9. The decrease in salivary flow produced by 0.5 mgm./kg. of the drug corresponded to that produced by 0.0025 to 0.005 mgm./kg. atropine sulfate.

10. Local irritation of the rabbit skin and of the rabbit eye was produced by solutions of the drug of 0.5 per cent strength or greater.

11. The local anesthetic effect of 1.0 per cent cocaine hydrochloride was dupli cated by 0.125 per cent 23B.

12. The drug had no effect upon the size of the pupil of the rabbit eye.

REFERENCES

1. Raymond, Albert L.: J. Am. Pharm. A., 32: 249–255, 1943.
2. Graham, J. D. P., and Lazarus, S.: This Journal, 69: 331–341, 1940.
3. Samaan, Karam, and Saad, Khalil: Quart. J. Pharm. & Pharmacol., 11: 679–691, 1938.
4. Behrens, Behrend: Arch. f. exper. Path. u. Pharmakol., 140: 237–256, 1929.
5. Barbour, Henry Gray: This Journal, 7: 547–555, 1915.
6. Geiger, E.: J. Am. Pharm. A., 29: 148–152, 1940.
7. Hazelton, Lloyd W., and Fortunato, Frank: J. Am. Pharm. A., 31: 60–64, 1942.
8. Grote, I. W., and Woods, Maribelle: J. Am. Pharm. A., 23: 266–270, 1944.
9. Gibbs, O. S.: J. Lab. and Clin. Med., 12: 686–694, 1927.

DIGITALIS V. THE BALJET REACTION AND PHARMACODYNAMICS OF DIGININ*

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Received for publication November 8, 1946

In 1937 Karrer (1) isolated a new glycoside from the leaves of *digitalis purpurea* and named the compound "diginin". The glycoside is crystalline and obtained in good yield; 5 to 10 grams per 100 Kg. of dried leaves. Diginin differs from the more common glycosides of digitalis, in that it is not a lactone. It is decomposed by dilute mineral acids into diginose and diginigenin. The genin appears to be a pregnane derivative (2).

Our attention was drawn to this glycoside because it was reported to give a positive Legal's reaction and yet have little cardiotonic activity. The presence of such a glycoside in digitalis in quantities comparable to the highly active cardiotonic glycosides appeared to be an obstacle to the recently developed chemical assay of digitalis (3 and 4) based upon the Baljet reaction. Accordingly we decided to investigate the glycoside pharmacodynamically and its behavior toward the Baljet reagent.

Properties of diginin. The diginin used in these studies was supplied by Dr. W. Karrer of Basle. The glycoside occurs in large, colorless well-formed crystals. It is very difficultly soluble in water, but readily soluble in alcohol and chloroform and less soluble in acetone and ethyl acetate. Its optical rotation is $(\alpha)_D^{20°} - 176°$ (37 per cent alcohol solution).

Hagemeier (5) has studied the Baljet reaction of twenty glycosides and genins including most of the important digitalis derivatives. He has also reported that the color intensity produced by diginin and the Baljet reagent was the highest of all the glycosidal substances he examined. No specific data are given for this substance but it is assumed that it was examined in an aqueous solution of very high dilution.

We have examined solutions of diginin in absolute methanol containing 5, 10, 15, 20, 25, and 30 mg./100 cc. by the method previously described (6). In Chart 1 typical time-optical density curves are given for diginin solutions of the six concentrations.

The curve for each concentration shows rapid color development within the first 10 or 20 minute period of the experiments and, in this respect, corresponds to the behavior of the other digitalis substances which we have examined under similar conditions. However, beyond this point the curves show a gradual increase in color intensity throughout the remainder of the 100-minute interval.

* The expense of this investigation was defrayed in part by a grant from the Board of Trustees of the United States Pharmacopoeial Convention.

The diginin used in these studies was kindly supplied us by Dr. Karrer of Basle through the Hoffman LaRoche Co. of Nutley, N. J.

The color intensities produced by other digitalis glycosides and genins reached a maximum value after 20 to 40 minutes and then either remained constant or showed a gradual decrease with time.

In Chart 2 the 30-minute optical density readings for each of the six solutions in Chart 1 have been plotted against the corresponding concentration. The locus of these points shows only a slight curvature and it is seen that the color produced by the diginin solutions in these experiments follows fairly closely the Beer-Lambert law. This observation is in marked contrast to the behavior of

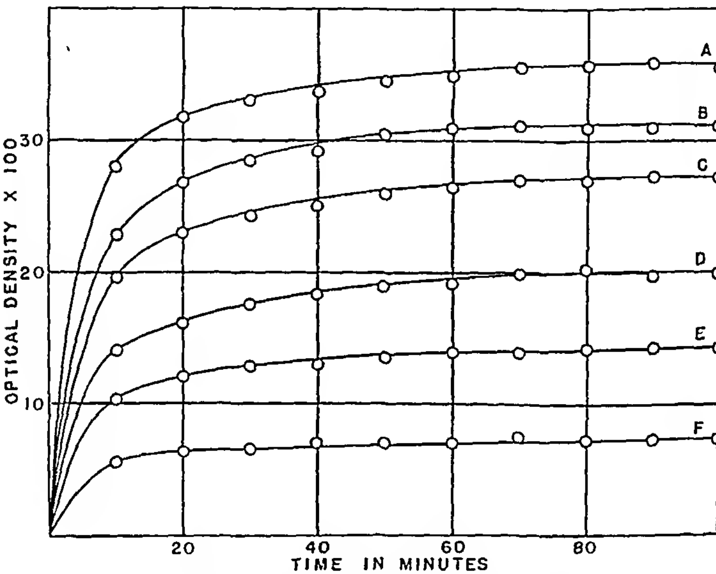

CHART 1. DIGININ SOLUTIONS IN ABSOLUTE METHANOL

A = 30 mg./100 cc.
B = 25 mg./100 cc.
C = 20 mg./100 cc.
D = 15 mg./100 cc.
E = 10 mg./100 cc.
F = 5 mg./100 cc.

the other digitalis principles under similar experimental conditions. This fact is shown very clearly in Chart 2 by comparing a typical digitoxin curve with the diginin curve. The curves in Chart 2 also show that, on a weight basis, the color intensities of the digitoxin solutions are approximately twice those of the corresponding diginin solutions. On the basis of information now available concerning the structure of diginin and if one assumes the presence of one mole of diginigenin for each mole of diginose then a molecular weight value of approximately 500 is obtained for the glycoside. Since digitoxin has a molecular weight of approximately 750, it is seen that if the curves in Chart 2 are plotted on the

basis of molar concentrations the diginin will show a color value approximately one-third of that of digitoxin.

Electrocardiographic studies (dog). Under ether anesthesia four dogs were given diginin and digitoxin intravenously and electrocardiographic tracings taken at various intervals. The solution of each glycoside was in 20 per cent alcohol made isotonic with sodium chloride. The digitoxin content was 0.1 mg./cc. and the diginin 0.2 mg./cc. In the first dog 200 cc. of the diginin solution were injected without alteration to the cardiac rhythm. When digitoxin was

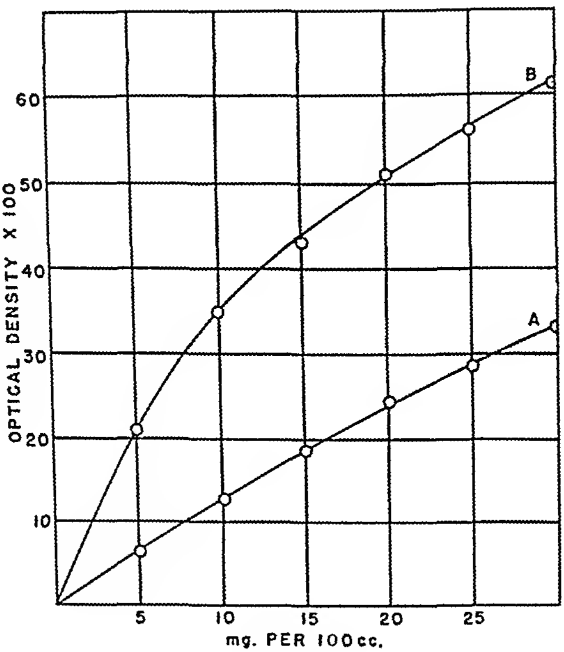

CHART 2
A = Diginin
B = Digitoxin

substituted for diginin solution the R-spike was depressed, the rate slowed, the polarity of the heart reversed and death was preceded by ventricular fibrillation.

In dog 2 (4.4 Kg.), digitoxin was injected—the animal died, after 44 cc. had been injected. The typical digitoxin effects Lead II are shown in Chart 3.

In dog 4 (4.7 Kg.) diginin 0.4 mg./cc. was injected, 20 cc. in 5 minutes and records were taken each 5 minutes until the animal died. One hundred and twenty cc. of diginin solution were required to produce death. Chart 4 shows the electrocardiographic tracing Lead II after 110 cc. and just prior to the death of the animal.

Perfused frog's heart. Fremherz (7) reported that diginin was comparatively weak in his tests on frogs by the typical assay procedures. This was observed

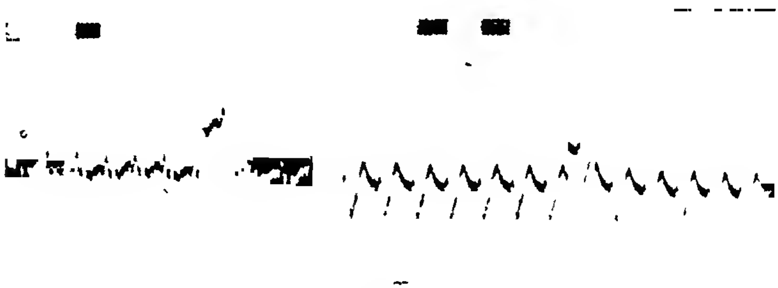

CHART 3

Dog #2—Normal Lead II
Female 4.4 Kg.

Lead II after 1 mg./Kg.
Digitoxin. Just prior to death.

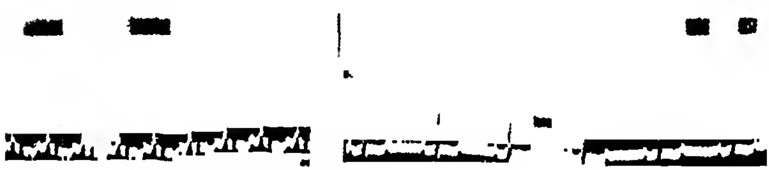

CHART 4

Lead II
Dog #4—Normal
Female 4.7 Kg.

Lead II after 10 mg./Kg
Diginin. Just prior to death.

CHART 5. PERFUSION WITH A SOLUTION OF 2.5 MG./100 CC. DIGITOXIN IN HOWELL-RINGER'S SOLUTION AT ARROW

Time in seconds.

by Chen (8). We compared the effects of diginin with those of digitoxin in 5 typical perfusion experiments *in situ*. The glycosides were dissolved in 50 per cent alcohol and diluted to a concentration of 5 mg./100 cc. with Howell-Ringer's

solution: in each instance the alcoholic content of the perfusion fluid was 5 per cent.

Typical effects of the two glycosides are shown in Charts 5 and 6.

Cat assay. We attempted to determine the lethal dose of diginin to the cat when the glycoside was injected by the U.S.P. digitalis assay procedure. In 7 animals no concordant value could be obtained. Using higher concentrations of the glycoside diginin, 2 animals succumbed at approximately 8 mg./Kg. No lethal dose could be attained in the other 5 animals. This was also observed by Chen (8).

Chart 6. Perfusion with a Solution of 5 mg./100 cc. Diginin in Howell-Ringer's Solution at Arrow

Perfusion with Howell-Ringer's solution resumed at second arrow with recovery. Time in seconds.

Discussion. In its behavior toward the Baljet reagent it is apparent that diginin assumes a unique position among all of the ditigalis principles which we have examined. On either a weight or molar basis, its solutions show the lowest color values that we have observed. It is the only digitalis glycoside we have examined for which the Baljet color reaction approximates the Beer-Lambert law. Although Hagemeier reported a maximum color intensity for diginin as compared with other digitalis substances it should be emphasized that his observations were probably made in aqueous solution and in concentrations were probably made in aqueous solution and in concentrations ten-fold more dilute than those used in our experiments.

The importance of this behavior of diginin with respect to our previously published methods for the assay of digitalis tinctures and digitoxin is self-evident. The assay value obtained by either method represents a summation of all of the chromogenic substances present regardless of their cardiotonic activity. We proceed with caution in interpreting the experimental results of this communication in terms of the much more complex conditions that prevail in the tincture assay.

Certain generalizations appear justifiable. All leaf preparations and their tinctures probably contain diginin. The error in the assay value owing to the presence of diginin will be dependent upon the relative amounts of this glycoside present in the sample being assayed and in the U.S.P. Reference Standard Digitalis. To account for the generally higher assay values obtained by the colorimetric method as compared with the official bioassay method on the basis of the presence of diginin, would require the assumption that most of the preparations assayed contained relatively more diginin that the U.S.P. Reference

Standard Digitalis. It appears probable that a gross imbalance in this diginin ratio would be required to produce a significant error in the final assay.

With regard to the colorimetric method of assay of digitoxin and its tablets, the effect of the possible presence of diginin as an impurity on the assay value obtained is rather obvious. In this case the experimental conditions of our diginin measurements closely parallel the assay procedure. The presence of diginin would lead to high assay values, but the error is of limited significance. Thus, assuming that the chromogenic values for diginin and digitoxin are additive in the experimental procedure employed then pure digitoxin contaminated with 5 per cent of diginin would yield a calculated assay value of approximately 97.5 per cent instead of the true value of 95 per cent.

Our pharmacodynamic studies on dogs, cats and the perfused frog's heart confirm the work of Fromherz and also Chen, on the injection of diginin into frogs, namely, that this glycoside exhibits little toxicity and manifests only a semblance of digitoxin activity.

On the perfused frog's heart produced only a slight diminution of the amplitude of beat, whereas digitoxin in the same concentration produced a rapid cardiac stoppage. In Chart 4 the effect of diginin on the dog's electrocardiogram is shown. The glycoside during the 30 minutes of injection gradually slowed the cardiac rate from 200 per minute to 100 per minute. The P-R interval was increased from 0.08 second to 0.12 second. The P-wave was depressed and the R-spike diminished about 30 per cent of its normal value. The T-wave remained positive. Depression of the S-T segment is present. Cardiac stoppage occurred without irregularity of rhythm or change in the electrical potential of the heart with 10 mg./Kg. of diginin in contrast to the digitoxin terminal cardiac picture with 1 mg./Kg.

Thus as diginin was found to be unique among the *purpurea* glycosides with respect to the Baljet reaction, it appears biologically unique in this group of cardiotonic principles. When sufficient quantities of the glycoside are available we propose to explore its therapeutic possibilities clinically.

SUMMARY

1. The reaction of diginin to the Baljet reagent has been studied and compared with the other glycosides of *digitalis purpurea*.

2. Diginin was found to give a very weak Baljet reaction and pharmacodynamically it appeared to be devoid of a typical digitoxin-like action on the heart.

REFERENCES

1. Karrer, W.: "Festschrift, Emil C. Barell." Friedrich Reinhardt A. G.: Basle. 238, 1936.
2. Shoppee, C. W., and Reichstein, T.: Helv. Chim. Acta. 23, 975, 1940.
3. Bell, F. K., and Krantz, J. C. Jr.: This Journal, 83, 213, 1945.
4. Bell, F. K., and Krantz, J. C., Jr.. This Journal, 87, 198, 1946.
5. Hagemeier, A.: Arch. expt. Path. Pharmakol. 194, 195, 1940.
6. Bell, F. K., and Krantz, J. C., Jr.: This Journal, 88, 14, 1946.
7. Ref. 5, page 202.
8. Chen, K. K., Personal communication 1946.

A METHOD OF SCREENING SYMPATHOMIMETIC AMINES FOR STIMULANT ACTION ON THE CEREBRUM

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Received for publication November 12, 1946

A need for a rapid method of screening a series of sympathomimetic compounds in order to compare possible stimulant action on the cerebrum to that of amphetamine and *d*-desoxy-ephedrine prompted the work contained in this report. The methods based on measuring increased activity of mice (1) and rats (2) did not appear selective enough since drugs like cocaine and caffeine also increase general activity. Selle's (3) observation of drooped wings, ataxia, excitement, and twittering in chicks that had received amphetamine led to the use of the chick as an experimental animal.

We tested numerous compounds selected to give a spectrum of pharmacological actions. In all, 60 compounds including the more commonly used sympathomimetic amines were tested.

Experimental. Chicks one day to five days old, chickens older than three weeks, squabs, pigeons, canaries, and day old ducklings were used. A solution containing 2–10 mgm. of a compound was injected beneath the skin of the axilla in all birds. In addition various combinations of drugs were tested as was bilateral extirpation of the cerebral hemispheres.

Results. Chicks react to amphetamine in a manner which is very easy to observe. The syndrome produced by injecting 2–5 mgm. of amphetamine into young chicks has three stages of development. The first two stages take place about one to two minutes after injection and consist of vigorous head movements, ruffled feathers and increased general activity. This is accompanied by a forward tilt of the body and drooping wings. The third stage, in the majority of cases, is experienced three minutes after injection. It is the crucial test for amphetamine action. Under the influence of the amphetamine, the ordinary intermittent peeping of the chick becomes a continous twitter, about 250 times per minute. At times the twittering even becomes a warble. This twittering reaction may last as long as one hour.

The appearance of corneal anaesthesia and a failure to respond when the toes and legs were pinched was noted at the height of the amphetamine action.

Among all the other compounds tested, some produced no reactions at all similar to the three stages of amphetamine, some produced parts of the three stages, and others showed almost opposite effects. Among the latter were synephrine and neo-synephrine which caused lethargy and a type of posture resembling that of a setting hen. Only *dl*-amphetamine, *d*-amphetamine, *d*-desoxyephedrine, beta-phenyl alpha amino butane, beta-phenyl tertiary butyl amine, consistently produced twittering along with the rest of the amphetamine syndrome.

TABLE 1

DRUG	MGM. PER CH	NO. CH	T	DW	E	C	L	F	OTHER EFFECTS
Acetanilid	2	5	0	0	0	0	0	0	
N-Acetylamphetamine	2	5	0	0	0	0	0	0	
Acetylcholine Chloride	2	5	0	0	0	0	0	0	Apnea, visible distension of blood vessels in legs, swelling, salivation, muscular weakness
Acetylsalicylic Acid	2–20	5	0	0	0	0	0	0	
Aminopyrine	2	9	1	0	0	0	0	0	
dl Amphetamine	2	20	20	20	20	7	0	1	Absence of corneal reflex, analgesia
d Amphetaminoaceto-piperidide	2	5	0	0	5	5	0	0	
Amphetaminoacetodi-ethylamide	2	5	0	0	0	5	0	0	
Amphetaminoacetodi-phenylmethylamide	2	5	0	0	0	0	0	0	
Antipyrine	2–10	7	0	0	0	0	0	0	
Arecoline HBr	2	5	0	0	0	5	0	5	Dyspnea
Atropine Sulfate	2	5	0	0	0	0	0	0	
Barium Chloride	2	5	0	0	0	5	0	0	
Benadryl	1–2	15	0	15	15	15	0	1	
Caffeine	2	5	0	0	0	0	0	0	
Cobefrine	2	5	0	0	0	0	5	5	Comatose
Cocaine	2	5	1	0	0	5	0	5	Startled chick
Curare (intocostrin)	2 U	5	0	0	0	0	0	5	Slow, shallow respiration
d-Desoxyephedrine	2	5	5	5	5	0	0	0	
Diethylaminoacetoam-phetamide	2	5	0	0	0	0	0	0	
Dilantin	2	5	0	0	0	1	1	0	
Ephedrine Sulfate	2	10	0	0	0	0	10	0	
Epinephrine HCl	0.05 0.5	7	0	0	0	0	7	0	Blanching of legs and toes, lethargic
Epinine HCl	2	5	0	0	0	0	0	0	
Ergotamine	0.1	5	0	1	1	0	4	0	Comatose
F 883	2	5	0	0	5	0	0	0	
F 933	2	5	0	0	0	0	5	0	Diarrhea
Guanidine HCl	2	5	0	0	0	0	0	0	
Histamine	0 2-5	6	0	0	0	0	0	0	Swelling feet
Hyoscine HBr	2	5	0	0	0	0	0	0	Spastic Gait
Insulin	16 U	5	0	0	0	0	0	0	
Mecholyl	2	5	0	0	0	0	0	1	Apnea, diarrhea, salivation
Metrazol	2	5	0	0	0	1	0	0	
Morphine	2	5	0	0	0	0	5	0	
Neo-synephrine	2	10	0	0	0	0	10	0	
Nicotine	2	5	0	0	0	5	0	5	

TABLE 1—*Concluded*

DRUG	MCM. PER CH	NO. CH	T	DW	E	C	L	F	OTHER EFFECTS
Papaverine	2	5	0	0	0	0	0	0	Apnea, salivation
Paredrine	2	5	2	5	5	0	0	0	
Pavatrine	0.2	5	0	0	0	0	0	0	
(Beta)phenylalpha-aminobutane	2	5	5	5	5	0	0	0	Identical with amphetamine
(Beta)phenylalpha-aminopentane	1–2	5	0	0	0	5	0	3	
Beta Phenyl tertiary butylamine	2	1	0	1	1	1	0	0	
	4	1	0	1	1	1	0	1	
	1	4	4	4	4	4	0	0	Twitter may not be as constant or as intense as amphetamine
Picrotoxin	2	5	0	0	0	5	0	0	
Pilocarpine	2	5	0	0	0	0	0	0	Paralysis, blindness, depression
Piperidinoaceto-amphetamide	2	5	0	0	0	0	0	0	
Piperidinoaceto-piperidide	2	5	0	0	0	0	0	0	
Physostigmine	2	5	0	5	0	5	0	5	
Procaine	2	5	0	0	0	0	5	0	Muscular weakness
Propadrine HCl	2	5	0	0	0	0	0	0	
Propylamphetamine	2	5	0	0	0	0	0	0	
Prostigmine	2	5	0	0	0	5	0	5	Lachrymation, apnea
Sodium bisulfite	3/200	5	0	0	0	0	0	0	
Strychnine	0.2	5	0	0	0	1	0	1	
Synephrine	2	19	0	0	0	0	0	0	
Trasentine	2	5	0	0	0	0	0	0	Muscular weakness
Tuaminoaceto-amphetamide	2	5	0	0	0	4	0	0	
Tyramine	2	5	0	0	0	0	0	0	

T = Twittering; CH = Chicks; DW = Drooped Wings; E = Excitement; C = Convulsions; L = Lethargy; F = Fatalities; U = Units.

Attempts to abolish the syndrome were made by using sympatholytic drugs (933 F, 883 F, beta-dibenzylaminoethyl-chloride), pentobarbital, and surgery. Only pentobarbital and bilateral extirpation of the cerebral hemispheres abolished the twittering. The latter did not abolish the other two stages.

Only chicks from one day to three weeks produced the twittering reaction with amphetamine. Squabs and hens ejected food as did pigeons which also lost their ability to fly. Canaries became convulsive and one day old ducklings showed excitement only.

The results from other compounds are listed in table 1 which lists the compounds in alphabetical order by names easily identified rather than by chemical nomenclature. It also lists the effects of each compound and the number of chicks so affected.

SUMMARY

Chicks are highly selective animals for screening compounds suspected of having a stimulant action similar to that of amphetamine because of a syndrome with a unique twittering reaction.

We wish to thank Dr. Louis S. Goodman of the University of Utah School of Medicine for the generous supply of Beta-dibenzylaminoethylchloride used in these experiments.

REFERENCES

1. Hauschild, F.: Arch. f. exper. Path. u. Pharmakol., 195: 647, 1940.
2. Schulte, J. W. et al.: J. Pharm. and Exp. Therap., 71: 62, 1941.
3. Selle, R.: Science, 91: 95, 1940.

STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS

II. Activity In Vitro of a Related Series of Ethers and Arylamines

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Received for publication November 15, 1946

The tuberculostatic activity of 2-butoxy 5-aminopyridine *in vitro* was described in a previous report (1). It was shown that this compound was capable of inhibiting the growth of virulent and avirulent tubercle bacilli in high dilution and that its bacteriostatic properties were specific for members of the genus *Mycobacterium*.

The acute toxicity of this substance (LD_{50}) for mice approximated 0.4 gram/kgm. (2). This rather high degree of toxicity interfered greatly with the application of this compound to studies on its effect in experimental tuberculosis *in vivo*. Nevertheless, by careful control of dosage it was shown that this pyridine derivative favorably influenced the course of tuberculosis in the guinea pig (2).

Because of its interesting properties it was decided to undertake a program of synthesis of various types of derivatives of 2-butoxy 5-aminopyridine with the object of preparing a compound with tuberculostatic properties but of lower toxicity for the animal host. The chemical work involving the synthesis of several hundred derivatives was carried out under the direction of one of us (H. L. F.). This report brings together some of the key changes in structure of the parent compound and presents data indicating how such changes in structure influence the tuberculostatic activity of the compound and its acute toxicity for mice.

Methods. *Tests in vitro:* The compounds were dissolved in Dorset's synthetic medium in serial concentrations ranging from 64 mg. per cent, by halves, to 0.008 mg. per cent. The inoculum, a suspension of the 607 strain of *M. tuberculosis*, was seeded in each series of tubes and was incubated for 96 hours. The lowest concentration of such a series which showed absence or almost complete inhibition of visible growth was recorded as the bacteriostatic concentration for each respective drug. Details of this procedure have been described previously (3). Where the symbol > is used before the bacteriostatic concentration, e.g., >4.0 mg. per cent, the compound was insoluble in concentrations greater than that indicated, and a saturated solution was not bacteriostatic.

Compounds that were basic in character, and which were frequently poorly soluble, almost always were used as the hydrochloride salts, but calculations of concentrations and amounts were always based on the equivalent amount of the free base.

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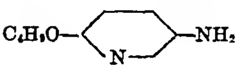
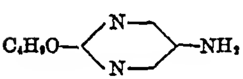
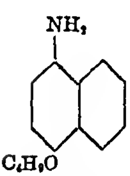
Toxicity tests: The acute toxicity tests were carried out in mice of both sexes; these animals ranged in weight from 19 to 21 grams and were dosed on the basis of the average weight, 20 grams. The mice were dosed either orally (P.O.) with a suspension or solution of the drug or subcutaneously (S.Q.) with a solution. Each dose was administered to a group of 3 animals and was varied from 5.0 gram/kgm., in decreasing quantities, to 0.5 gram/kgm. The mice were then observed for a period of 7 days and deaths which occurred within that time were recorded. This procedure is adequate only to determine the approximate range of toxicity and the results recorded in the tables are based on the following key:

TABLE 1

Cyclic Isosteres

	TBC. STASIS	ACUTE TOXICITY—P.O.
	mgm. per cent	
	0.063	4+
	0.031	4+
	0.063	3+
	2.0	3+

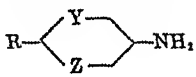
4+ = LD_{50} is 0.5 gram/kgm. or less
3+ = LD_{50} between 0.5 and 1.25 gram/kgm.
2+ = LD_{50} between 1.25 and 2.5 gram/kgm.
1+ = LD_{50} between 2.5 and 5.0 gram/kgm.
0 = LD_{50} at some dose greater than 5.0 gram/kgm.

RESULTS. *Cyclic isosteres:* To test the essentiality of the pyridine ring for tuberculostatic activity, the benzene isostere of 2-butoxy 5-aminopyridine was synthesized. This compound, p-butoxyaniline, was found to have practically the same degree of activity and toxicity as its pyridine counterpart. As is shown in table 1, the pyrimidine isostere was similarly active. However, the naphthalene ring, with the amino and butoxy groups in the 1- and 4-positions, respectively, was considerably less active, although its toxicity was only slightly, if at all, reduced.

Alkoxy derivatives: Several compounds were synthesized in which the length of the alkoxy chain was varied from 1 to as many as 16 carbon atoms; some branched chain structures were also prepared. These compounds are shown in table 2 and include both benzene and pyridine derivatives. The positions of the

TABLE 2

2-Alkoxy derivatives



R	Y	Z	TBC. STASIS	ACUTE TOXICITY—P.O.
			mgm. per cent	
CH_3O—	C	N	32.0	2+
CH_3O—	C	C	5.0	3+
C_2H_5O—	C	N	4.0	2+
C_2H_5O—	C	C	0.5	3+
C_3H_7O—	C	N	0.25	4+
C_4H_9O—	C	N	0.031	4+
C_4H_9O	C	C	0.063	4+
C_4H_9O	N	N	0.125	3+
iso C_4H_9O—	C	N	0.5	3+
sec C_4H_9O—	C	N	2.0	3+
$C_5H_{11}O$—	C	N	0.031	4+
$C_5H_{11}O$—	C	C	0.063	
iso $C_5H_{11}O$—	C	N	1.0	4+
iso $C_5H_{11}O$—	C	C	1.0	
$(C_2H_5)_2CHO$—	C	N	0.063	3+
$C_6H_{13}O$—	C	N	0.031	4+
$C_6H_{13}O$—	C	C	0.031	4+
$C_6H_{13}O$—	N	N	0.063	
$C_7H_{15}O$—	C	N	0.5	3+
$C_8H_{17}O$—	C	N	4.0	
$C_8H_{17}O$—	C	C	8.0	
$C_{10}H_{21}O$—	C	N	16.0	2+
$C_{10}H_{21}O$—	C	C	>4.0	
$C_{16}H_{33}O$—	C	C	>4.0	
Substituted Alkoxy Derivatives				
HOOC—CH_2CH_2O—	C	C	>64.0	
$(C_2H_5)_2$—N—CH_2CH_2O—	C	C	>64.0	
$(C_2H_5)_2$—N—CH_2CH_2O—	C	N	>64.0	

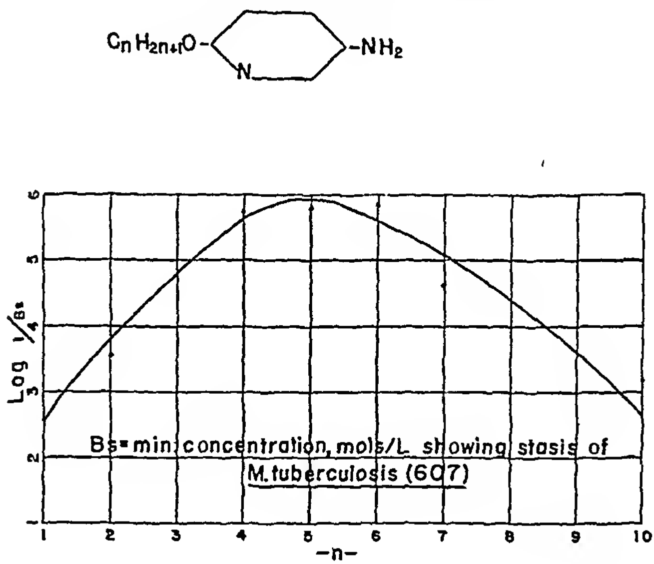
amino and alkoxy groups were kept para to each other throughout. The data in this table indicate that the very short chain compounds such as methyl and ethyl and the long chain derivatives were much less active than the compounds in which the alkoxy substituents consisted of 4, 5 or 6 carbon atoms. Moreover, if the chain was branched, the activity was considerably reduced. The sub-

stituted alkoxy compounds were found to be inactive. Also, it should be noted that in the compounds in which the alkoxy group consisted of a very long or a very short chain the toxicity was somewhat reduced, in comparison with the more active compounds in which the chain was of intermediate length.

Figure 1 illustrates the effect of the number of carbon atoms in the alkoxy group upon the bacteriostatic activity against the tubercle bacillus. The number of carbon atoms is plotted along the abscissa and the logarithm of the reciprocal of the lowest tuberculostatic concentration is plotted along the ordinate.

FIGURE 1.

2-ALKOXY DERIVATIVES



The resulting curve illustrates the variation of activity with the number of atoms in the alkoxy chain.

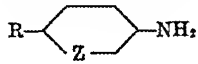
Other derivatives in which variations in the original alkoxy group have been introduced are shown in table 3. The acyclic group was replaced by cyclic structures including some heterocyclic as well as isocyclic derivatives. In most cases the compounds were of greatly reduced activity or were inactive.

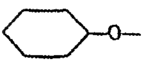
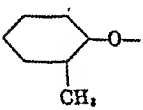
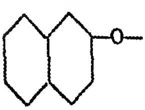
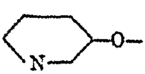
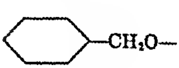
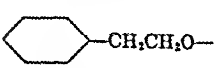
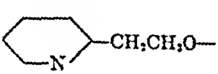
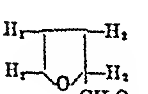
Isomers: Isomers of the original compound, as well as of other ring structure analogues, were prepared and the data are shown in table 4. On the whole, variations in the relative positions of the amino and alkoxy groups in the pyridine nucleus resulted in inactive compounds. In the pyrimidine nucleus also, it was found that the 2-butoxy 5-amino compound was active, whereas 2-amino 4-butoxy was inactive. Among the benzene compounds it was found that although

substitution of groups in positions para to each other resulted in the most active substances, substitutions in the meta positions also yielded active compounds,

TABLE 3

Cyclicoxy and Cyclicalkoxy



R	Z	TBC. STASIS	ACUTE TOXICITY—P. O.
		mgm. per cen	
	N	4.0	4+
	C	>4.0	2+
	N	16.0	3+
	N	8.0	
	N	64.0	2+
	N	0.5	
	N	4.0	3+
	C	4.0	
	N	64.0	
	N	4.0	2+

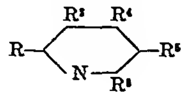
but the activity was not as marked. When the groups were situated ortho to each other no activity was found. It is interesting to note that the toxicity,

wherever it was determined, was as high in the inactive as in the active compounds.

Nuclear substitutions: Compounds in which another radical, methyl, nitro, amino or butoxy, was substituted on the ring nucleus were synthesized only

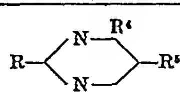
TABLE 4

Isomers



R	R[3]	R[4]	R[5]	R[6]	TBC. STASIS	ACUTE TOXICITY P.O.	ACUTE TOXICITY S.O.
					mgm. per cent		
C_2H_5O—	H	H	H	NH_2	>64.0		
C_4H_9O—	NH_2	H	H	H	32.0		
C_4H_9O—	H	H	H	NH_2	32.0		
$C_5H_{11}O$—	H	H	H	NH_2	>4.0		
C_4H_9O—	H	H	NH_2	H	0.031	4+	4+

		R[2]	R[3]	R[4]			
CH_3O—		NH_2	H	H	>64.0	3+	
C_2H_5O—		NH_2	H	H	64.0		
C_4H_9O—		H	NH_2	H	0.25	4+	
C_4H_9O—		H	H	NH_2	0.063	4+	4+



			R[4]	R[5]			
NH_2—			C_4H_9O	H	32.0		
C_4H_9O—			H	NH_2	0.125	3+	

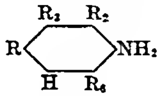
in the benzene series; such substitutions resulted in inactive compounds. These data are shown in table 5.

Ether replacement: To determine whether the ether group is essential, derivatives were made in which alkyls replaced the alkoxy substituents. These data are shown in table 6. In the benzene series, at least, aklyl compounds were almost as active as the analogous alkoxy derivatives (table 2) indicating that linkage through oxygen is not essential to activity. However, the toxicity of the alkyl compounds is as high as is that of compounds with ether linkages.

Other variations in the ether group: Compounds were synthesized in which a second and a third ether linkage were added to the alkoxy group of the parent substance. Table 7 gives the activities of such compounds. The examples shown evidence decreasing activity as the addition of alkoxy groups tend to increase the molecular weight of the compound. It may be significant however, that the methoxy-ethoxy, and the ethoxy-ethoxy derivatives still retain some activity, while the toxicity of these compounds seems to be significantly reduced. It is interesting to note the inactivity and the low toxicity of the hy-

TABLE 5

Nuclear Substitutions



R	R_3	R_2	R_6	TBC. STASIS
				mgm. per cent
$CH_3O—$	H	$NH_2—$	$CH_3—$	>4.0
$C_2H_5O—$	NO_2	H	H	>8.0
$C_4H_9O—$	H	NH_2	H	>64.0
H	H	$C_4H_9O—$	$C_4H_9O—$	>4.0

TABLE 6

Ether Replacement Alkyl Derivatives

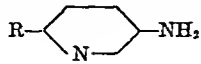
R	TBC. STASIS	ACUTE TOXICITY—P. O.
	mgm. per cent	
Cl	8.0	
$C_2H_5—$	0.25	3+
$C_4H_9—$	0.063	
$C_6H_{13}—$	0.25	4+
$C_8H_{17}—$	8.0	

droxyethoxy derivative (table 7), since the analogous ethoxy compound, listed in table 2, has a minimal bacteriostatic concentration of 4.0 mg. per cent and has a fair degree of toxicity.

Amino replacement compounds: The essential nature of the amino group of the parent substance is demonstrated by data shown in table 8. Elimination of the amino group results in a complete loss of activity, and replacement of it by a carboxyl group, hydroxy or ethoxy group has the same effect. Replacement with a nitro radical sharply reduces activity, although some activity remains. Possibly, this may be accounted for by a partial reduction by the growing organism of the nitro group to an amino group.

TABLE 7

2-Alkoxy-alkoxy and 2-Aryloxy-alkoxy Derivatives



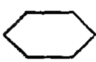
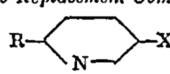
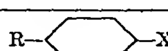
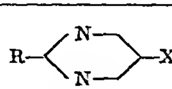
R	TBC. STASIS	ACUTE TOXICITY—P. O.
	mgm. per cent	
HOC_2H_4O—	64.0	0
$CH_3O—C_2H_4O$—	1.0	1+
$C_2H_5O—C_2H_4O$—	0.5	2+
$C_4H_9O—C_2H_4O$—	4.0	4+
$C_2H_5O—C_2H_4O—C_2H_4O$—	64.0	
—$CH_2O—C_2H_4O$—	32.0	3+
—$O—C_2H_4O$—	16.0	3+

TABLE 8

Amino Replacement Compounds



R	X	TBC. STASIS
		mgm. per cent
C_4H_9O—	H	64.0
C_4H_9O—	NO_2	4.0
$C_5H_{11}O$—	NO_2	8.0
C_4H_9O—	COOH	64.0
		
C_4H_9O—	H	64.0
C_2H_5O—	C_2H_5O—	64.0
C_2H_5O—	OH	32.0
		
C_4H_9O—	NO_2	0.25

Amino substitutions: A number of compounds was synthesized in which one or both of the hydrogens of the amino group in the parent substance, were replaced by a variety of substituents. In table 9 the first 2 compounds represent the original parent pyridine compound and its benzene isostere; these are included for reference purposes. Since it was found that the greatest activity is obtained in the compounds in which the alkoxy group consists of 4, 5 or 6 carbon atoms, the 4-carbon-atom-compound is to be used as the point of reference for the various substitutions on the amino group. The replacement of one of the hydrogens on the amine by a sodium sulfite radical completely destroys the activity but also reduces the toxicity. The activity is for the most part only partially reduced by the substitution of an alkyl, carboxymethyl or hydroxyethyl substituent, but a replacement of both hydrogens of the amine by a methyl, results in a further reduction of bacteriostatic effect. The toxicity is not reduced by any of these changes.

The substitution of an acyl in the amino group produces compounds which are still highly active but the benzoylamino derivative is not active. It is particularly interesting to note that the monocarboxylic acyls reduce the toxicity of the parent substance slightly, if at all, whereas, succinyl, phthaloyl and phthalimid derivatives greatly reduce toxicity without much loss in activity *in vitro.* The apparent effect on toxicity may be due to poor absorption or to rapid excretion.

Derivatives of carbamic acid are represented, but there are too few to permit generalizations with respect to their activity, since they include compounds of considerable activity as well as inactive compounds. The sulfonamide and the methyl sulfonyl compounds showed very low activity; in the case of the former the toxicity was negligible.

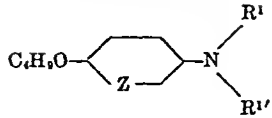
A series of compounds involving sodium formaldehyde sulfoxylate, sodium formaldehyde bisulfite and sodium acetaldehyde bisulfite substitutions on the amino group was next described. These compounds exerted activity similar to that of the parent substance, especially in the group with a pyridine nucleus. Further, the toxicity of the pyridine derivatives was significantly less than that of the parent compounds, although the same modifications in the benzene series did not lead to a similar decrease in toxicity.

The hydrogens of the amino group were also substituted by glucose bisulfite, glucose, anil, and, benzaldehyde anil, procedures which resulted only in slight loss of activity but which had little effect on toxicity.

Discussion. The character of the bacteriostatic activity of the active compounds comprising this report is exemplified by 2-butoxy 5-aminopyridine. In an earlier report (1), it was shown that the action was one of growth inhibition rather than bactericidal effect, and that this effect was specific for members of the genus *Mycobacterium.* It is of interest that virulent strains of human tubercle bacilli were susceptible to much lower concentrations of this substance (1 to 100,000,000) than was the avirulent rapidly growing strain 607 (1 to 3,000,000). We are not aware of any other substance that shows such remarkable specificity for one genus of microbiological organism. The degree

TABLE 9

Amino Substitutions



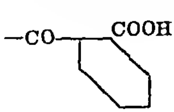
Z	R^1	$R^{1\prime}$	TBC. STASIS	ACUTE TOXICITY P.O.	ACUTE TOXICITY S.Q.
	Unsubstituted				
			mgm. per cent		
N	H	H	0.031	4+	4+
C	H	H	0.063	4+	4+
	Inorganic substituents				
C	$-SO_3Na$	H	4.0		
N	$-SO_3Na$	H	4.0	1+	
	Acyclic substituents				
C	$-CH_3$	H	0.125	4+	
N	$-CH_3$	CH_3	4.0		
N	$-C_4H_9$	H	0.5	4+	
C	$-CH_2COOH$	H	0.25	4+	4+
N	$-CH_2COOH$	H	0.125	4+	
N	$-CH_2CH_2OH$	H	0.125	4+	
	Acyls, acyclic and cyclic substituents				
N	$-COCH_3$	H	0.063	3+	
N	$-COC_3H_7$	H	0.125	3+	2+
N	$-COCH_2CH_2COOH$	H	0.25	1+	
N		H	0.125	1+	2+
N		H	>4.0		
N			1.0	0	0

TABLE 9—*Continued*

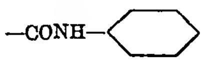
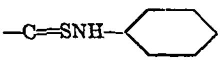
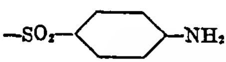
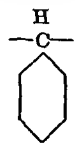
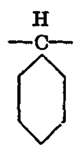
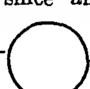
Z	R^1	$R^{1'}$	TBC. STASIS	ACUTE TOXICITY P.O.	ACUTE TOXICITY S.Q.
	Miscellaneous substituents				
			mgm. per cent		
N	$—CONH_2$	H	16.0	1+	
N		H	>16.0		
N		H	1.0	1+	
N	$—C{=}NH \cdot NH_2$	H	0.125	3+	3+
N		H	4.0		
N		H	>4.0	0	
	Sodium formaldehyde sulfoxylate derivatives				
C	$—CH_2SO_2Na$	H	0.125	3+	3+
N	$—CH_2SO_2Na$	H	0.125	1+	
	Sodium formaldehyde bisulfite derivatives				
C	$—CH_2SO_3Na$	H	0.125	4+	
N	$—CH_2SO_3Na$	H	0.063	0	2+
C	$—CH_2SO_3Na$	CH_3	2.0	4+	
	Sodium acetaldehyde bisulfite derivatives				
C	$—CH \cdot CH_3 \cdot SO_3Na$	H	2.0		
N	$—CH \cdot CH_3 \cdot SO_3Na$	H	0.5	3+	
	Glucose bisulfite derivatives				
N	SO_3Na $—C(CHOH)_3CHCH_2OH$ └——O——┘	H	0.125	3+	2+
C	SO_3Na $—C(CHOH)_3CHCH_2OH$ └——O——┘	H	0.125	3+	

TABLE 9—*Concluded*

Z	R^1	$R^{1'}$	TBC. STASIS	ACUTE TOXICITY	
				P.O.	S.Q.
Glucose anils					
			mgm. per cent		
C	—CH(CHOH)$_3$CHCH$_2$OH └——O——┘	H	0.125		
N	—CH(CHOH)$_3$CHCH$_2$OH └——O——┘	H	0.063	3+	2+
Benzaldehyde anil					
C			>4.0	4+	
N			0.125	3+	2+

of stasis is unsurpassed even by antibiotics such as streptomycin, which in our experience inhibits the growth of pathogenic strains and of the 607 strain in dilutions no greater than 1 to 3,000,000. The activity of streptomycin however, is bactericidal in concentrations approaching its bacteriostatic range.

Data illustrating the absorption, excretion and suppressive activity in tuberculosis of guinea pigs of some of the more active compounds of this series are in preparation and will be presented shortly.

The structural specificity of the compounds comprising this series is quite marked, since almost any variation from the parent structure, i.e. C_nH_{2n+1}—O——NH_2, diminishes or destroys activity. It has been possible to retain activity in the benzene series by exchanging alkoxy for alkyl, and in both benzene and pyridine series by certain substitutions on the amino group.

Isosteres, within the limited number synthesized, retained almost all the activity of the parent compound. This parallels what has been found in other cases; one cycle may be replaced by another with retention of certain biological properties (e.g., sulfapyridine, sulfathiazole, sulfadiazine). The naphthalene compound is not a true isostere and its reduced activity may be accounted for by considering it to be a di-nuclear substituted compound.

The curve of activity, as related to the length of the alkoxy chain (figure 1), is typical of the changes in activity in an homologous series (e.g., substituted resorcinols and phenylethylamines), in which the peak of activity is reached with certain chain lengths. In the present series of compounds the same length of a substituent chain gives peak activity in each of the isosteric groups. Branched chains reduce the activity markedly. It is not clear why 2°-butyl alcohol has such lowered activity while 2°-pentyl alcohol (3-pentyloxy derivative) retains the activity of the n-pentyl chain, since both are straight chain secondary substituted radicals. Substitution of a hydrogen by an —OH (table 7), by a —COOH, or by a —NEt_2 group (table 2) results in a complete loss of activity.

The para-position appears to be important for high activity. The considerable activity displayed by ortho-butoxy aniline (table 4) indicates, however, that the para-position is not absolutely essential. In the case of the nitrogen-heterocycles, the tautomeric form of the —NH_2 group must also be considered; it appears that an imino group lacks activity.

Although nuclear substituents in general destroy the activity, perhaps too few examples are available to warrant a definite conclusion.

As is shown in table 6, the ether linkage in the benzene nucleus group may be eliminated, by substitution with alkyl, without serious loss of activity. However, the essential character of the ether linkage in the pyridine group of compounds can only be contemplated until examples are available. Compounds with replacements of the 2-alkoxy group by —COOH, —SO_3H, or SO_2NH_2 etc., which are not included in this report, are inactive.

The most interesting compounds from the point of view of retention of activity with reduction in toxicity were found in the group in which one or both hydrogens of the amino radical were replaced (table 9). That a similar reduction in toxicity was not manifested by the same compounds in the benzene group cannot be explained. In contrast to the sulfonamides, many other substitutions may be made in the —NH_2 group without markedly diminishing bacteriostatic activity. The acetyl derivative is of interest, since its possible formation, *in vivo*, would not result in the inactivation of the compound.

SUMMARY

2-Butoxy 5-aminopyridine exerts high and specific bacteriostatic action on *Mycobacteria*.

Variations in the structure of this compound indicate that:

1. isosteres retain activity;
2. the number of carbon atoms in the alkoxy group is optimally 4 to 6;
3. cyclic substitutions in the alkoxy linkage diminish activity; other substitutions in the alkoxy group that tend to increase the molecular weight of the compound or which involve branched chains also result in compounds of greatly diminished activity;
4. in general, only specific isomers are active;
5. nuclear substitutions result in diminution of activity;

6. the ether linkage of the alkoxy group may be replaced by alkyl, in the benzene isostere series, without a serious loss in activity;

7. the amino group is essential to activity;

8. when the amino group is substituted by certain inorganic or acyclic substituents, activity is diminished but not abolished;

9. the amino group may be substituted by acyl, sodium formaldehyde bisulfite, sodium formaldehyde sulfoxylate and certain other aldehydic groupings without serious loss in activity in most cases. This type of substitution results in compounds with decreased toxicity in the pyridine series, but does not produce a marked change in toxicity in the series of benzene isosteres.

The pharmacological and experimental therapeutic activity of several of the most effective and least toxic of the compounds should be investigated.

REFERENCES

1. Feinstone, W. H.: Proc. Soc. Exp. Biol. and Med. 63: 153, 1946.
2. *To be published.*
3. Fitzgerald, R. J., and Feinstone, W. H.: Proc. Soc. Exp. Biol. and Med., 52: 27, 1943.

PHARMACOLOGICAL PROPERTIES OF A NEW ADRENERGIC BLOCKING AGENT: N,N-DIBENZYL-β-CHLOROETHYLAMINE (DIBENAMINE)[1,2]

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Received for publication November 26, 1946

Chemical agents blocking specific biological systems or functions are of value in many fields of physiological investigation and in therapeutics. Notable examples of such agents include cyanides, atropine and related alkaloids, paraminobenzoic acid and thiourea derivatives. In the past much effort has been expended to develop drugs which would adequately block the sympatho-adrenal system. Ergot alkaloids were first studed for this purpose (1). Subsequently, yohimbine and ethyl yohimbine (2), certain benzodioxane derivatives, 933F, 883F, etc., benzyl-imidazoline (Priscol), and many other substances have been reported to possess some degree of adrenergic blocking activity. However, the usefulness of the aforementioned compounds is limited by their toxicity, side-effects (such as the pressor and oxytocic actions of ergot alkaloids and the direct depressor action of Priscol (3)), or short duration and incompleteness of blocking action.

The present report deals with the properties of a new sympatho-adrenal blocking agent, N,N-dibenzyl-β-chloroethylamine (Dibenamine) which appears to have the advantages of specificity, potency and prolonged action. Preliminary reports on the pharmacological properties of Dibenaminc have been presented (4, 5). The relationship of chemical structure to adrenergic blocking activity has also been briefly reported (6) and will be fully presented in a subsequent publication.

PREPARATION AND PHYSICAL PROPERTIES. N,N-dibenzyl-β-chloroethylamine and its salts with organic and inorganic acids are about equally active, but the hydrochloride salt was used in most experiments because it is easier to handle than the free base which is an oily liquid. The hydrochloride salt is a white, crystalline substance, practically insoluble in water near neutrality, but soluble in acid (2% at pH 2.1, 1% at pH 2.4 and 0.5% at pH 2.7), in 95% ethanol and in propylene glycol. It is stable in acid solution, but rapidly loses activity in neutral or alkaline aqueous solutions. A 10% stock solution may be prepared by dissolving the hydrochloride salt in propylene glycol or 95% ethanol made slightly acid (0.05 N) with concentrated H_2SO_4. Such stock solutions are very stable. Immediately prior to administration, the stock solution was diluted 1:10

[1] Trade mark of Givaudan-Delawanna, Inc.

[2] This investigation was aided by a grant from Givaudan-Delawanna, Inc Dr. W. Gump of this company kindly supplied Dibenamine hydrochloride and related compounds used in this study.

or 1:20 with 0.9% NaCl solution. The acid in the stock solution was found adequate to keep the drug in solution after dilution. Dibenamine was administered intravenously, intraperitoneally, intramuscularly, subcutaneously and orally, and was found to exert its systemic effects by all these routes of administration. Dibenamine was given orally in solid form, in gelatin capsules or in lactose tablets.

Blocking of adrenergic pressor effects. The direct effect of Dibenamine intravenously on the normal arterial pressure is variable. Rapid injection in anesthetized animals may cause an abrupt and marked fall in arterial pressure which may be lethal. Smaller doses or slower rates of injection produce either a slight fall or rise in pressure, the latter occuring particularly after a previous dose of Dibenamine. However, with sufficiently slow injections (10 to 20 minutes in anesthetized cats; 45 to 60 minutes in recumbent humans) full adrenergic blocking doses of Dibenamine may be administered intravenously with only slight changes in the normal blood pressure.

It is of interest to compare the effect on blood pressure of complete sympathectomy (7, 8) with that of adrenergic blocking by Dibenamine. After sympathectomy there is a sudden drop in blood pressure followed by a gradual return to normal, indicating that after recovery from the trauma of the operation the vascular musculature is capable of an adjustment which allows for the maintenance of a normal blood pressure in the absence of sympathetic nerve impulses. After Dibenamine this adjustment apparently can be made without an intervening period of hypotension.

Some degree of adrenergic blocking is evident within 5 to 10 minutes after minimal effective doses of Dibenamine administered intravenously and somewhat earlier with larger doses, but the maximal blocking action is not exerted until at least 30 minutes after injection. The duration of the adrenergic block from a single injection varies from approximately 36 hours to a maximum of 5 days. Preliminary observations in man (9), and in animals with experimental renal hypertension, indicate that residual therapeutic effects may last as long as seven to ten days.

A. Injected epinephrine. After administration of Dibenamine the usual pressor response to epinephrine is changed to a pure depressor response (fig. 1). In contrast to results obtained with certain other blocking agents, this reversal phenomenon is independent of the anesthetic used. Comparable results were obtained in animals under pentobarbital, urethane or cyclopropane anesthesia, in animals with a high spinal cord section, and in unanesthetized animals and man.

The adrenolytic effect of Dibenamine is more complete than that usually produced by other blocking agents. For example, after adequate doses of Dibenamine in anesthetized cats (10 to 20 mgm./kgm.), the pressor effects of even massive intravenous doses of epinephrine are completely reversed. In figure 2 is illustrated the response to 1000 μgm./kgm. of epinephrine, and ten times this dose has been administered with comparable results. The depressor response to massive doses of epinephrine is essentially the same in magnitude but

is more prolonged than that to much smaller doses. As can be seen in both figures 1 and 2, the pulse pressure is increased during the period of epinephrine-induced hypotension.

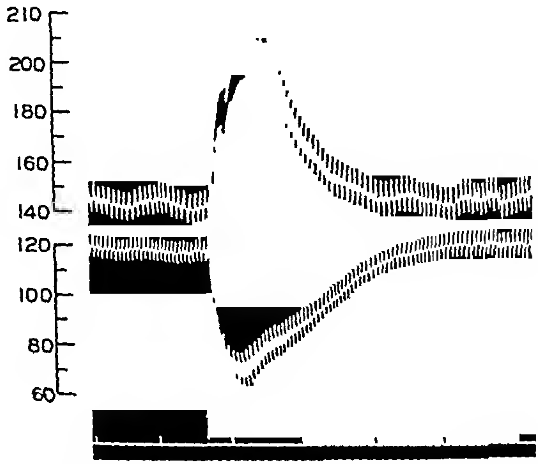

FIG. 1. EFFECT OF DIBENAMINE ON THE BLOOD PRESSURE RESPONSE TO A SMALL DOSE OF EPINEPHRINE

Upper record before and lower record after administration of 15 mgm./kgm. Dibenamine to a cat anesthetized with pentobarbital. The arrow indicates intravenous injection of 2.5 μgm./kgm. epinephrine. Time in minutes.

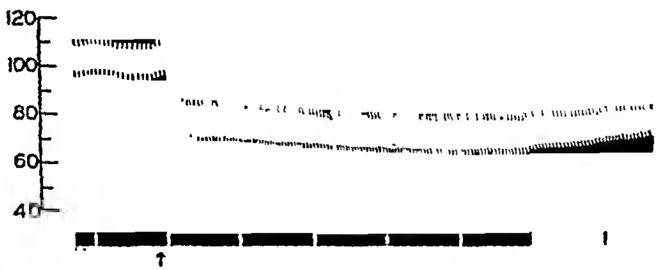

FIG. 2. EFFECT OF DIBENAMINE ON THE BLOOD PRESSURE RESPONSE TO A LARGE DOSE OF EPINEPHRINE

At the arrow 1000 μgm./kgm. of epinephrine was injected intravenously. Time in minutes.

The reversal by Dibenamine of the pressor response to epinephrine probably represents a blocking of the vasoconstrictor action and a consequent unmasking of the inhibitory vasodilator action of epinephrine. Vasodilatation occurs in

response to small doses of epinephrine in the absence of blocking agents (*see* Dale (10)), but ordinarily it is obscured by the more powerful vasoconstriction. It is of interest to observe that in the rabbit, an animal in which Cannon and Lyman (11) were unable to find any vasodepressor action of epinephrine, Dibenamine largely abolishes the pressor effect of epinephrine but does not cause a reversal. Presumably there is no depressor component to be unmasked.

The magnitude of the depressor response to epinephrine after Dibenamine tends to vary directly with the initial blood pressure level, but it is not strictly dependent upon the latter. The depressor action of epinephrine after ergotamine and yohimibine disappears completely when the blood pressure falls below 40 to 60 mm. Hg (12); in contrast, definite reversals occasionally have been obtained after Dibenamine with initial pressures as low as 30 mm. Hg. The depressor response to epinephrine after Dibenamine varies in different animals from 20 to 60 mm. Hg or more. Even a relatively small dose of epinephrine (1.0 to 2.5 μgm./kgm.) elicits an almost maximal response. This fact suggests that there is a definite limit to such sympathomimetic vasodilatation and that maximal hypotension is elicited by an amount of epinephrine much smaller than that producing maximal hypertension (fig. 3).

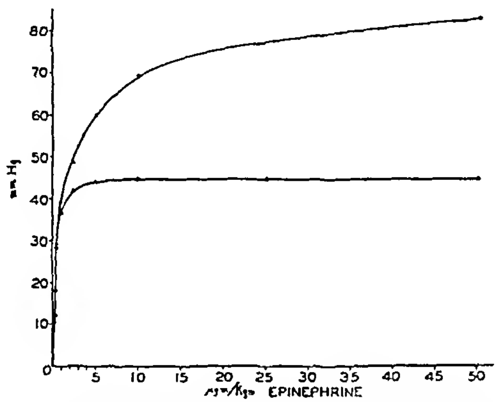

FIG. 3. RELATION OF THE DOSE OF EPINEPHRINE (INTRAVENOUS) TO THE HYPERTENSION (●—●) ELICITED BEFORE AND THE HYPOTENSION (▲—▲) ELICITED AFTER THE ADMINISTRATION OF DIBENAMINE

Averaged data from 6 cats.

The increase in heart rate and pulse pressure induced by epinephrine are not prevented by Dibenamine. Although cardiac output was not measured directly, the combination of tachycardia, increased pulse pressure, and hypotension (indicating a decreased peripheral resistance) indicates that both the stroke volume and the cardiac output are increased by epinephrine after blocking with Dibenamine. Both the tachycardia and the positive inotropic effect on the myocardium (indicated by the increased stroke volume) probably represent

direct actions of epinephrine on cardiac muscle. The inability of Dibenamine to block these two actions may indicate a fundamental difference in the mechanism of action of epinephrine on smooth as compared to cardiac muscle.

Blocking by Dibenamine of the pressor response to epinephrine is usually complete, *i.e.*, there is a pure depressor response to all doses of epinephrine. It is quite difficult to select a dose of Dibenamine which produces an incomplete block. However, when the action is wearing off (in cats, usually two to four days after injection) a partial block may be observed. During this period, small doses of epinephrine produce a brief pressor response before the depressor effect appears, and large doses of epinephrine produce an almost pure pressor response.

B. *Splanchnic nerve stimulation.* The action of Dibenamine on the vasopressor response to sympathetic nerve stimulation was studied in cats. Both pentobarbital and urethane anesthesia were employed with essentially similar results. The splanchnic nerves on one side were cut and shielded electrodes placed on the distal stumps just above the diaphragm. Stimulation was applied by means of a variable frequency, square-wave electronic stimulator with an output current largely independent of the external resistance (Woodbury and Nickerson (13)). A frequency of 20/second and a pulse width of 0.5 millisecond were utilized, and the current necessary for a maximal pressor response in the untreated animal was determined. Responses were recorded before and after treatment with Dibenamine and finally after extirpation of the adrenal glands. Currents several times the control strength were also used after Dibenamine, but no alteration in the response or "breaking through" could be demonstrated. There appeared to be no significant alteration in the excitability of the splanchnic nerves as a result of the administration of Dibenamine.

Prolonged (45 seconds) unilateral stimulation of the splanchnic nerves in an untreated animal causes a sharp, biphasic rise in blood pressure which frequently amounts to 60–80 mm. Hg (fig. 4A). The initial rapid component of the rise is the result of direct sympathetic constriction of the splanchnic vessels, and the subsequent rise (developing after 20 to 25 seconds) is due to the release of epinephrine, and probably some sympathin, into the systemic circulation. This secondary rise is largely eliminated by removal of the adrenal glands or by their exclusion from the circulation.

After treatment with Dibenamine, the blood pressure rise from splanchnic stimulation is converted into a biphasic fall (fig. 4B). The initial rapid fall is rather small and may be absent in experiments in some animals. This would seem to indicate either that sympathetic vasodilatation in the splanchnic bed is limited in cats or that compensatory mechanisms not blocked by Dibenamine are operating. The second component of the reversal is much more pronounced and is comparable in duration to the hypotension produced by the injection of 2.5 μgm./kgm. of epinephrine.

It was found more difficult to block and reverse the pressor component due to the local action of sympathetic nerve impulses than the pressor component due to epinephrine, either endogenous or injected. In figure 5 is illustrated the result of

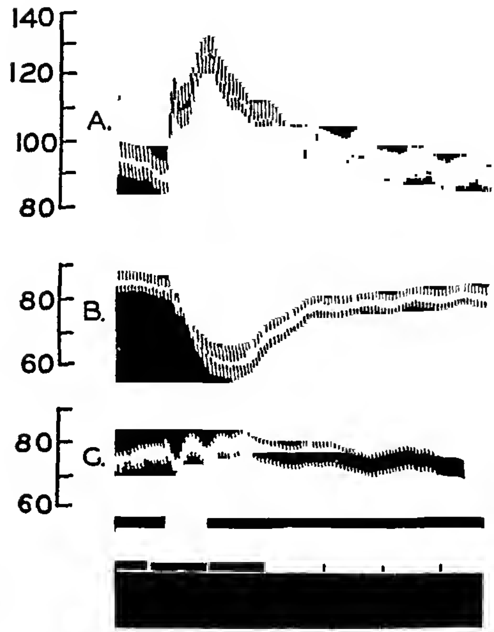

FIG. 4. BLOOD PRESSURE RESPONSE TO SPLANCHNIC NERVE STIMULATION

(A) before and (B) after the administration of Dibenamine, and (C) after removal of the adrenal glands.

Cat under urethane anesthesia. Electrodes on the distal stumps of the resected right splanchnic nerves just above the diaphragm. Period of stimulation indicated by upward deflection of signal line. Time in minutes.

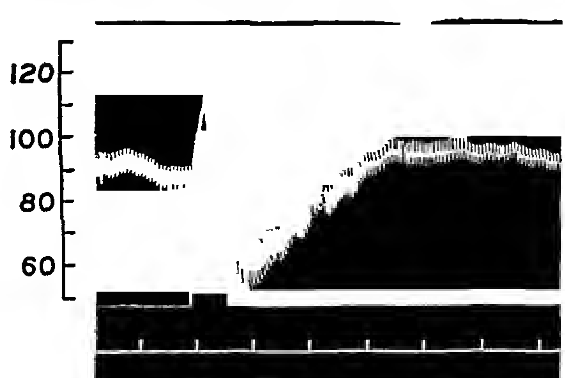

FIG. 5. BLOOD PRESSURE RESPONSE TO SPLANCHNIC NERVE STIMULATION AFTER INCOMPLETE ADRENERGIC BLOCK WITH DIBENAMINE

Cat under urethane anesthesia. Electrodes, etc. as in legend to fig. 4.

splanchnic nerve stimulation after incomplete adrenergic blocking by Dibenamine. The neurogenic pressor response is essentially unaltered, but the epinephrine-induced hypertension is completely reversed.

After removal of the adrenal glands in an animal with a complete adrenergic block, splanchnic stimulation may cause a slight fall or no change in the blood pressure (fig. 4C). In some experiments, a slow pressor response may appear after 20 to 30 seconds of stimulation, *i.e.*, during the period when epinephrine would ordinarily be released into the circulation. A moderate increase in heart rate (15 to 20%) accompanies this blood pressure rise. A similar pressor effect was noted after ergotoxine by Cannon and Rosenblueth (14) who attributed it to an increase in heart rate produced by sympathin E released into the circulation

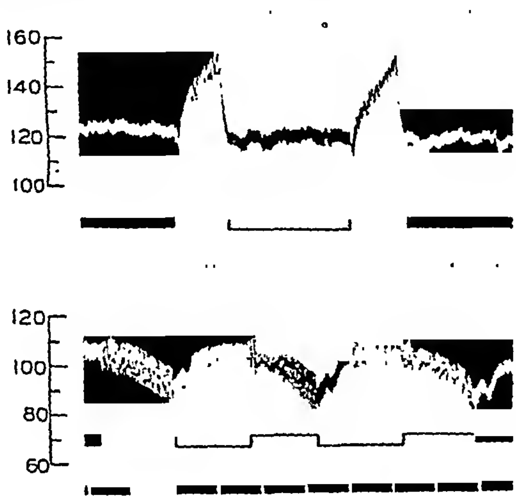

FIG. 6. BLOOD PRESSURE RESPONSE TO ANOXIA BEFORE (UPPER RECORD) AND AFTER (LOWER RECORD) DIBENAMINE

Cat under pentobarbital anesthesia. Periods of anoxia indicated by upward deflections of signal line. Time in minutes.

and not blocked by ergotoxine. Apparently insufficient sympathin I is released by splanchnic stimulation to overcome the pressor effect of the cardioacceleration. A similar explanation may apply to the delayed pressor response to splanchnic stimulation in adrenalectomized animals treated with Dibenamine, as the drug does not block the tachycardia elicited by epinephrine or sympathin E.

C. *Reflex adrenergic pressor effects.* The effect of Dibenamine on reflexly induced sympathetic vasoconstriction was also studied. Gradually developing anoxia was produced in both cats and dogs by the use of a small rebreathing bag and a soda-lime canister. A typical series of blood pressure records from such an experiment is shown in figure 6. After Dibenamine, the usual pressor response to short periods of anoxia is converted into a pure depressor response.

Another method employed to test the ability of Dibenamine to block and reverse the pressor effects of a generalized sympathetic discharge was to observe the blood pressure response to the nicotinic action of choline esters in atropinized animals. Results with carbaminoylcholine and with acetylcholine (after eserine) were essentially the same. Two such experiments on cats under pentobarbital anesthesia are illustrated in figure 7. After the slow intravenous administration of 1 mgm./kgm. of atropine sulfate, 150 μgm./kgm. of carbominoylcholine was injected rapidly intravenously, causing the typical biphasic pressor response shown in figure 7A (upper record). After the administration of 15 mgm./kgm. of Dibenamine, the same dose of carbaminoylcholine produced the depressor response illustrated in figure 7A (lower record). Similar experiments were per-

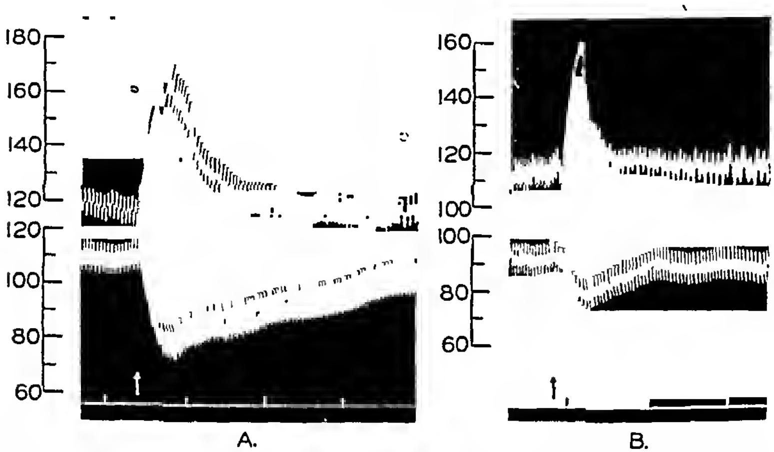

FIG. 7. BLOOD PRESSURE RESPONSE TO THE INTRAVENOUS ADMINISTRATION OF 150 μGM./KGM OF CARBAMINOYLCHOLINE IN AN ATROPINIZED CAT UNDER PENTOBARBITAL ANESTHESIA

A. Upper record before and lower record after the intravenous administration of 15 mgm./kgm. of Dibenamine.

B. The same procedures as in A, after bilateral adrenalectomy. Injections of carbaminoylcholine indicated by arrows. Time in minutes

formed on animals after bilateral adrenalectomy. The pressor response is characteristically less prolonged (fig. 7B, upper record), and the depressor response after Dibenamine (fig. 7B, lower record) is never as great as with the adrenals intact.

In these experiments it is reasonable to assume that all the sympathetic postganglionic fibers are stimulated by the massive doses of choline esters employed. The results indicate that Dibenamine is capable of reversing the pressor effects of a widespread discharge of the sympathetic nervous system, both in the presence and in the absence of the adrenal glands.

Dibenamine does not modify the fasciculation and respiratory embarrassment caused by the direct nicotinic action of the choline esters on skeletal muscle.

Sympathetic nervous system activity in excitement or rage is known to have a

marked effect upon the blood picture in certain animals. Izquierdo and Cannon (15) and Menkin (16) reported that excitement caused a sharp increase in erythrocyte count and a relative increase in mononuclear leukocytes in normal but not in sympathectomized cats. The blood changes were shown to be due primarily to splenic contraction induced by the sympatho-adrenal discharge which accompanies excitement.

Freely flowing blood from an ear vein was obtained from cats at rest and two minutes after they had been forcibly held on their backs and allowed to struggle for five minutes. Duplicate tests were performed on each of two animals before and 24 to 30 hours after the subcutaneous administration of 50 mgm./kgm. of Dibenamine. All four experiments gave essentially the same results which are averaged in table 1. These results indicate that Dibenamine is effective in blocking reflexly induced splenic contraction.

EFFECT ON OTHER SYMPATHETIC FUNCTIONS. Several objective signs of sympathetic blocking by Dibenamine are apparent in the intact animal. In

TABLE 1

Blood picture of control and Dibenamine-treated cats before, and after 5 minutes of struggle

CONTROL	BEFORE STRUGGLE	AFTER STRUGGLE
Erythrocyte count	8,990,000	12,030,000
Leukocyte count	10,075	14,000
Mononuclear cells	28%	39%
TREATED WITH DIBENAMINE		
Erythrocyte count	9,540,000	9,660,000
Leukocyte count	11,050	10,125
Mononuclear cells	18%	14%

cats, the drug produces a marked extension of the nictitating membrane, ptosis and some decrease in the mydriasis occurring in dim light. The last-named effect is one of the first signs of Dibenamine activity in man and appears after doses which do not appreciably affect the response to injected epinephrine. After Dibenamine, the iris in cats and rabbits shows considerable movement in response to changes in illumination. This movement is probably largely due to alterations in cholinergic tone. After both sympathetic and parasympathetic blocking the iris occupies an intermediate position which is independent of illumination. Data averaged from four experiments on cats are given in table 2. The position of the iris after blocking both constrictor and dilator impulses is probably a measure of the relative inherent tone of the two sets of muscle fibers.

Mydriasis, widening of the palpebral fissure and retraction of the nictitating membrane due to epinephrine or electrical stimulation of the cervical sympathetic nerve are also abolished or greatly reduced by Dibenamine. This action of Dibenamine is in sharp contrast to that of Priscol which was reported by Chess and Yonkman (17) to be ineffective in abolishing the mydriasis induced by

epinephrine or electrical stimulation of the cervical sympathetic nerve. In addition, the exaggerated responses of the previously (10 to 25 days) denervated iris and nictitating membrane to injected epinephrine are eliminated by Dibenamine. As a result of sensitization by denervation, the iris exhibits maximal mydriasis and the nictitating membrane maximal retraction in response to doses of epinephrine of less than 1 μgm./kgm. intravenously; after blocking with Dibenamine, the iris and nictitating membrane are unresponsive to at least 100 μgm./kgm.

The action of Dibenamine on the pilomotor response to sympathetic nerve stimulation was determined in cats anesthetized with pentobarbital or urethane. Electrodes were placed on the abdominal sympathetic chains just anterior to the sacral promontory. Square wave stimulation with a frequency of 20/second and a pulse width of 0.5 millisecond was applied, and the current required to produce a maximal pilomotor response in the tail determined. After Dibenamine (15 mgm./kgm., intravenously), the stimulation was repeated and the current gradually increased. Even with currents 10 times as great as the control, no pilomotor response was obtained. Currents stronger than this could not be used because the spread of current from the shielded electrodes produced a vigorous motor response in the hind legs and tail.

TABLE 2

Horizontal diameter of cat pupils before and after autonomic blocking

	LIGHT*	DARK†
	mm.	*mm.*
Control..	1	9.2
After Dibenamine...	1	6.0
After Dibenamine and atropine. .	7.3	7.4

* 60-watt lamp with reflector, 8 inches from eye.

† Just sufficient light to read a millimeter rule while measuring pupils.

Dibenamine provides marked protection against the lethal effects of epinephrine, both mice and rats being completely protected against more than twice the $L.D._{100}$ of epinephrine injected intraperitoneally. The results obtained are even more striking when it is considered that Dibenamine prevents local vasoconstriction by epinephrine and consequently the amine enters the circulation more rapidly.

It has been suggested (18) that the lethal effect of large doses of epinephrine is due to a direct action on the myocardium. If this were the case, the protective action of Dibenamine would be difficult to explain as this agent does not prevent the known effects of epinephrine on cardiac muscle, *i.e.*, the increase in rate and strength of contraction. In our experiments, death from epinephrine injected intraperitoneally appears to be respiratory. Terminally, respirations become labored, anoxic convulsions ensue and breathing ceases at a time when the heart is still beating. After the intraperitoneal administration of one or two $L.D._{100}$

of epinephrine to control rats, the survival period may be increased from less than 10 minutes to one to two hours by the use of adequate artificial respiration. At the end of this period pulmonary edema is so marked that oxygen exchange appears to be impossible and artificial respiration is no longer effective. In addition to pulmonary edema there may be a factor of central or reflex (carotid chemoreceptor (19)) respiratory inhibition involved in rapid deaths from large doses of epinephrine, but our present data are inadequate to allow a definite evaluation of this possibility.

Hamilton, Woodbury and Vogt (20) demonstrated a marked increase in both arterial and venous pulmonary pressures in unanesthetized dogs after large doses of epinephrine; these pressure changes are largely secondary to the increased pressure in the systemic circulation. It may be that the protection afforded by Dibenamine against the lethal effects of epinephrine is due to prevention of the rise in systemic blood pressure and the consequent deleterious changes in the pulmonary circulation.

Dibenamine also prevents cardiac irregularities induced by epinephrine in animals under cyclopropane anesthesia. As reported by Nickerson *et al.* (21), the intravenous injection of 10 μgm./kgm. of epinephrine in dogs equilibrated to 30% cyclopropane in oxygen produces ventricular fibrillation and death in a large percentage of animals and long periods of ventricular extrasystoles, tachycardia and other ventricular irregularities in those which survive. After Dibenamine (20 mgm./kgm., intravenously), epinephrine does not elicit ventricular tachycardia or fibrillation, and other arrhythmias are almost completely eliminated. However, the sinus tachycardia induced by epinephrine is not prevented. Dibenamine affords protection even when 1000 μgm./kgm. of epinephrine are injected. It is more effective than any other drug tested, including atropine, isonipecaine, ergotamine, procaine and Priscol. These studies will be published later in full.

Sympatho-adrenal discharges or injected epinephrine usually produce a sharp rise in blood glucose. To determine the effect of Dibenamine on this phenomenon, fasted rabbits were injected subcutaneously, with 0.2 mgm./kgm. of epinephrine and the blood sugar followed at intervals by a modification of the method of Hagedorn and Jensen. Typical curves obtained before and 20 hours after injecting 50 mgm./kgm. of Dibenamine subcutaneously are shown in figure 8. Dibenamine produces no significant inhibition of the epinephrine-induced rise in blood glucose; indeed, a more rapid rise and fall in blood sugar are consistently present. After Dibenamine, epinephrine no longer limits its own absorption by local vasoconstriction, and consequently it more readily enters the circulation, as reflected in the more rapid changes in blood glucose.

Dibenamine does not block the marked hyperventilation induced by the injection of epinephrine.

ACTION IN EXPERIMENTAL RENAL HYPERTENSION. The theoretical basis for the use of adrenergic blocking agents in experimental renal hypertension is not firmly established and the published work regarding the therapeutic effectiveness of such agents is not conclusive. For example, Alpert *et al.* (22) and Freeman and

Page (23) have reported that sympathectomy may cause a variable lowering of the blood pressure in dogs with renal hypertension but it does not prevent the development of the syndrome. In dogs with renal hypertension, Jacobs and Yonkman (24) demonstrated a slight hypotensive effect with chronic oral administration of large doses of yohimbine but this effect was not correlated with the degree of blocking produced by the drug.

Preliminary observations indicate that Dibenamine may be effective in lowering the blood pressure in rats with renal hypertension produced by compressing the kidneys according to the technic of Grollman (25). A response to the oral administration of Dibenamine is shown in figure 9. The decrease from the hypertensive level is marked and persists for several days after the last dose of Dibenamine. Studies are being continued to determine more exactly the extent

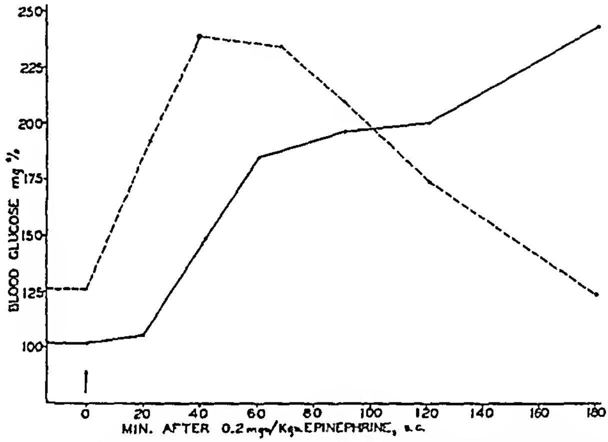

FIG. 8. BLOOD SUGAR RESPONSE TO INJECTED EPINEPHRINE (ARROW) IN A FASTED RABBIT BEFORE (●—●) AND 20 HOURS AFTER (●——●) THE SUBCUTANEOUS ADMINISTRATION OF 50 MGM./KGM. OF DIBENAMINE

to which Dibenamine is capable of lowering the blood pressure in chronic renal hypertension.

ACTION ON THE INHIBITORY EFFECTS OF EPINEPHRINE. The results reported above indicate that Dibenamine blocks many of the "excitatory" effects of epinephrine and of sympathetic nerve stimulation. In discussing the fall in blood pressure caused by epinephrine after Dibenamine, it was assumed that the drug exerted no blocking action on the "inhibitory" effects of epinephrine (*i.e.*, vasodilatation). This assumption is supported by more direct observations. When tested *in vitro* for its action upon spontaneously contracting small intestine (rat and rabbit), Dibenamine had no effect on the relaxation produced by epinephrine. Intestine obtained from animals previously treated with Dibenamine also showed a normal response to epinephrine. Epinephrine-

induced relaxation of the non-pregnant cat uterus *in situ* was also unaffected by Dibenamine in doses which completely reversed the blood pressure response

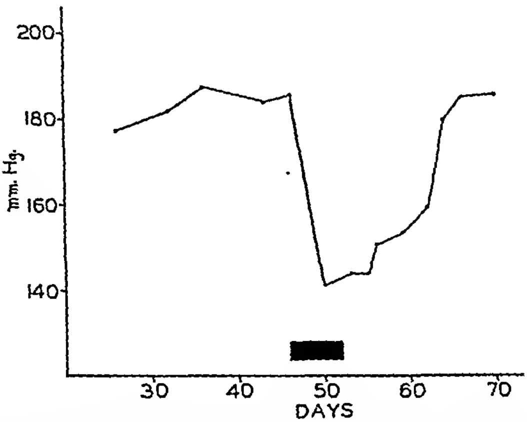

FIG. 9. BLOOD PRESSURE RESPONSE OF A RENAL-HYPERTENSIVE RAT TO THE ORAL ADMINISTRATION OF 75 MGM./KGM./DAY OF DIBENAMINE

Black rectangle indicates period of treatment. Time in days after compressing second kidney.

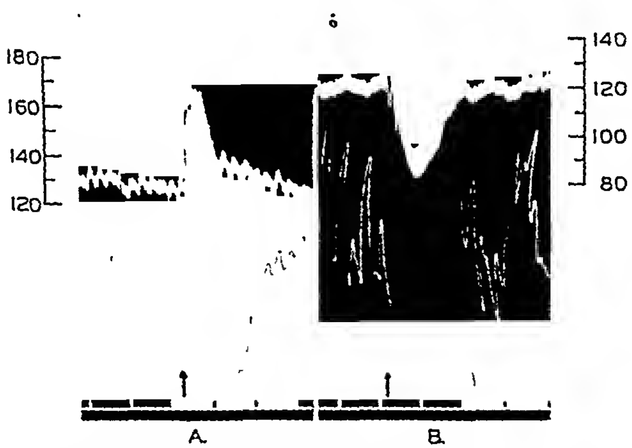

FIG. 10. RESPONSE OF THE BLOOD PRESSURE AND THE NON-PREGNANT CAT UTERUS IN SITU TO EPINEPHRINE (A) BEFORE AND (B) AFTER THE INTRAVENOUS ADMINISTRATION OF 15 MGM./KGM. OF DIBENAMINE

Downward deflections in the lower tracing indicate uterine relaxation. The arrows indicate the intravenous injection of 2.5 μgm./kgm. of epinephrine. Time in minutes.

(fig. 10). Although Dibenamine does not prevent uterine relaxation in response to epinephrine, it is capable of preventing and reversing the epinephrine-induced contraction of the non-pregnant rabbit uterus, both *in vivo* and *in vitro*.

TOXICITY. The toxicity of Dibenamine is manifested in two different ways, in local tissue damage and in central nervous system stimulation. It is closely related chemically to the nitrogen mustards (alkyl di-β-chloroethylamines and *tri-β-chloroethylamine), and although much less reactive than the latter, it is* capable of causing considerable local tissue destruction. This property is reflected in the high toxicity of Dibenamine after intraperitoneal administration. The L.D.$_{50}$ for mice by this route is 75 to 100 mgm./kgm. as compared to 400 to 500 mgm./kgm. when given subcutaneously. Death from intraperitoneal administration of doses near the L.D.$_{50}$ does not occur for one to seven days and is apparently due to local injury to the intestines and other abdominal viscera.

Rapid intravenous injection of Dibenamine leads to coordinated clonic convulsions which may recur intermittently over a period of a few minutes to an hour or more. Consciousness apparently is retained as indicated by the persistence of cortical righting reflexes and grasping efforts during the convulsion. Respiration is markedly stimulated. When Dibenamine is given rapidly intravenously, the convulsive dose is approximately 25 mgm./kgm. for cats and 35 mgm./kgm. for mice. Doses of 35 mgm./kgm. in cats and 50 mgm./kgm. in mice are frequently lethal within less than one hour.

The central excitatory effect is apparently independent of the adrenergic blocking action of Dibenamine. Its congener, N,N-dibenzyl-aminoethanol, has no blocking acitivity but manifests a comparable convulsant action. Also, central excitation due to Dibenamine appears earlier and disappears much sooner than the adrenergic blocking action.

The L.D.$_{50}$ of intravenously administered Dibenamine varies greatly with the speed of injection. When the entire dose is given to mice within less than one minute, the L.D.$_{50}$ is approximately 50 mgm./kgm. In contrast, doses as high as 300 mgm./kgm. are tolerated when given over a period of thirty minutes or more. In general, animals survive intravenous doses of almost any size if acute excitation of the central nervous system is avoided. The tolerated dose is greatly increased by slow injection or prior sedation with barbiturate. The highly variable injurious local reaction after intraperitoneal administration and the marked variation in central action with different rates of intravenous injection make accurate determinations of the L.D.$_{50}$ by these routes difficult.

The effective blocking dose of Dibenamine is much more constant than the toxic dose. The latter varies considerably with different routes and technics of administration, and this is reflected in variations in the therapeutic index. For example, the therapeutic index for protection of mice against the lethal effects of 10 mgm./kgm. of epinephrine administered intraperitoneally is 7 to 10 when Dibenamine is given intraperitoneally; about 40, subcutaneously; and from 5 to 50, intravenously, depending upon the speed of injection. These figures support the conclusion that the adrenergic blocking action and toxic effects of Dibenamine are not intimately related, a matter of importance in developing methods of administration which permit an adequate margin of safety.

The nitrogen mustards selectively attack dividing cells and consequently exert a toxic action on bone marrow and lymphoid tissues (26, 27, 28). Lymphopenia, granulocytopenia, thrombocytopenia and even aplastic anemia may be

produced, and the effect on hemopoiesis is usually fully developed within a few days. In the range of tolerated doses, however, Dibenamine does not affect the bone marrow. Rats injected subcutaneously with as much as three times the adrenergic blocking dose daily for four months showed a normal blood picture at the end of the experiment except for a mild leukocytosis, probably in response to local irritation from the subcutaneous administration. Less prolonged tests with closely related compounds (N-ethyl-N-benzyl-β-chloroethylamine and N,N-di(p-methylbenzyl)-β-chloroethylamine) also failed to produce bone marrow damage. It seems probable that at least two β-chloroethyl substitutions in the amine are necessary to produce bone marrow injury.

Dibenamine exhibits only slight cumulative toxicity. Daily subcutaneous injections of three times the adrenergic blocking dose given to growing rats for a period of four months produce no permanent ill effects and only a slight decrease in the growth rate during the period of administration. The recovery of susceptibility to epinephrine is complete approximately five days after the drug is discontinued.

Locus of action of dibenamine. On the basis of experiments described above and certain additional observations, tentative conclusions may be drawn as to the mechanism and locus of action of Dibenamine as an adrenergic blocking agent. One possible mode of action would be for Dibenamine to alter or promote the alteration of epinephrine so that the inhibitory effects would predominate. In connection with their extensive observations on adrenergic neuroeffector systems, Cannon and Rosenblueth (14) suggested that ergotoxine might act to modify epinephrine in such a way that it could no longer effectively combine with the E substance in effector cells. Morison and Lissak (29) were able to show that 933F caused a three-fold increase in the rate of oxidative destruction of epinephrine *in vitro*, and they interpreted the limited adrenergic blocking action of 933F as due, at least in part, to an increased rate of destruction of epinephrine *in vivo*. Although the $>N-CH_2CH_2Cl$ grouping is capable of reacting with many substances, four different lines of evidence indicate that Dibenamine does not produce its adrenergic blocking and reversing action by a direct alteration or destruction of epinephrine and sympathin.

(1). Dibenamine does not promote the destruction of epinephrine *in vitro*. It is possible to test directly the activity of epinephrine in mixtures with Dibenamine due to the somewhat delayed action of the latter. Control solutions without Dibenamine and mixtures of 10 moles of Dibenamine to 1 mole of epinephrine were incubated at 38°C and at room temperature at pH 7.4. After 20 hours all the solutions exhibited a decreased epinephrine potency as measured by pressor activity in anesthetized cats, but the solutions containing Dibenamine uniformly showed a greater pressor potency than the controls (an average of 45% potency in solutions with Dibenamine at 38°C compared to 21% for the controls, and 75% potency in solutions with Dibenamine at room temperature compared to 30% for the controls). The protection of epinephrine afforded by Dibenamine is undoubtedly due to the release of HCl during its decomposition in an alkaline medium with a slight lowering of the pH of the solution.

(2). Dibenamine blocks and reverses the effects of epinephrine over a very

wide dose range. Intravenous doses of epinephrine ranging from 0.1 μgm./kgm. to 10 mgm./kgm. (a range of 100,000 times) are completely blocked and reversed. It is difficult to conceive of Dibenamine promoting a sufficiently rapid and complete alteration of massive doses of epinephrine so that no excitatory action of the unaltered amine can be detected.

(3). The duration of action of a given dose of epinephrine, either endogenous or injected, is essentially the same before and after the administration of Dibenamine, as is apparent in figures 1 and 3.

(4). The blocking action persists after the Dibenamine has apparently been inactivated or excreted. More complete data on this point will be presented in a subsequent communication.

Another possible mode of action would be for Dibenamine to promote the activation by epinephrine of cholinergic or histaminergic vasodepressor mechanisms. However, in numerous experiments atropine, Benadryl, and Pyribenzamine were found to be completely ineffective in altering the vasodepressor response to epinephrine or sympathetic nerve stimulation in animals given Dibenamine. This was true in cats and dogs, although in the latter Bülbring and Burn (30) found the "sympathetic" vasodilators to be largely cholinergic.

The experiments reported above do not offer any evidence for an alteration by Dibenamine of the release of sympathin at postganglionic nerve endings or of epinephrine from the adrenal medulla. Although qualitatively modified by Dibenamine, the circulatory responses to electrical stimulation of the splanchnic nerves (fig. 4) and to cholinergic stimulation of sympathetic ganglia (fig. 7) are not significantly altered in duration. These experiments as well as others on the reversal of the anoxic rise in blood pressure also indicate that Dibenamine has no significant action upon nerve or ganglionic transmission.

Stimulation of the distal portion of the cut splanchnic nerves in adrenalectomized cats given Dibenamine causes vasodepression and tachycardia, indicating that both sympathin E and I are being liberated. This points to a locus of action of Dibenamine somewhere between the site of release of sympathin and the effector cells, probably directly on the latter. The mechanism by which Dibenamine prevents the excitatory response of effector cells to epinephrine and sympathin E is unknown at present. The block may be in some reaction essential for the excitation of effector cells by epinephrine or sympathin E. Practically nothing is known of such a reaction, but the long duration of action of Dibenamine suggests that some substance required for this reaction is destroyed or inactivated and that it is only slowly replaced or reactivated. Such an interpretation finds an analogy in the action of di-isopropyl fluorophosphate in destroying cholinesterase (31). In both cases there is a similar prolonged action, but in the case of Dibenamine the substance acted upon is completely unknown. Perhaps further studies of the blocking mechanism of Dibenamine will provide an understanding of the steps involved in the excitation of smooth muscle cells by epinephrine and sympathin.

Discussion. The experiments reported above indicate the usefulness of Dibenamine as a tool for studying the function of the sympatho-adrenal system.

The drug also possesses characteristics which may permit its therapeutic use for blocking the effects of sympatho-adrenal activity. Dibenamine has a high specificity of action, and its primary side-effects (local tissue destruction and central excitation) can be largely eliminated by a proper selection of routes and rates of administration. The adrenergic block produced is unusually complete and prolonged, a matter of importance from a therapeutic as well as an experimental standpoint. Preliminary observations on the human pharmacology of Dibenamine (9) indicate that its actions in man closely parallel those in amimals.

Numerous clinical conditions come to mind in which Dibenamine might be anticipated to exert a beneficial effect. For example, peripheral vascular diseases in which there is a component of functional spasm might be benefited by blocking the effects of sympathetic vasoconstrictor impulses. Raynaud's disease, early Buerger's disease (32), causalgic states (33, 34), and the vascular spasm accompanying embolism and thrombophlebitis (35) respond favorably to the interruption of sympathetic nerve impulses. Preliminary studies indicate a definite and prolonged salutary effect of Dibenamine in Raynaud's disease. The treatment of polycythemia rubra vera by interference with sympathetic vasoconstriction is less well established, but it has an experimental basis in the work of Schafer (36).

An adrenergic blocking agent might also have specific usefulness in such cases of hypertension as may be due to hyperfunction of the sympatho-adrenal system. The potential value of sympathetic blocking agents in essential hypertension is less obvious,.but it is suggested by the beneficial results obtained from total or sub-total sympathectomy (37, 38, 39). The preliminary observations on the effect of Dibenamine in experimental renal hypertension are also quite suggestive. A few cases of human essential hypertension given this drug showed an initial transient orthostatic hypotension and a more prolonged lowering of the blood pressure, but the response has been variable.

Under certain conditions a blocking agent such as Dibenamine might be expected to have advantages over the methods currently employed to interrupt sympathetic nerve impulses. Its more prolonged action would constitute an advantage over paravertebral local anesthetic block and ganglionic block with tetraethylammonium compounds (40, 41). Advantages over sympathectomy would include an action on all vascular beds, avoidance of sensitization to epinephrine or sympathin, and the maintenance intact of such vasodilator fibers as may be present in the sympathetic pathways. Finally, Dibenamine would have the important advantage over surgical resection of controlled reversibility.

The drug is now being studied by a number of investigators for its possible clinical value, and their studies should provide answers to some of the questions raised in this discussion.

SUMMARY

Studies on the pharmacology of N,N,dibenzyl-β-chloroethylamine (Dibenamine) revealed the following:

1. The primary toxic effects of Dibenamine are local tissue damage and central

excitation. When central excitation is prevented by slow administration or sedation, large intravenous doses are well tolerated.

2. Dibenamine produces its systemic effects when administered orally, subcutaneously, intramuscularly, intraperitoneally or intravenously.

3. Dibenamine blocks and reverses the vasopressor response to all doses of epinephrine investigated (0.1 μgm. to 10 mgm./kgm). This adrenergic blocking action may persist for three to four days after a single injection of Dibenamine.

4. Dibenamine does not prevent an increase in heart rate and cardiac output in response to epinephrine.

5. Dibenamine blocks the excitatory responses of smooth muscle to sympathetic nerve stimulation and to epinephrine, as shown by:

(a) Reversal of the vasopressor response to electrical stimulation of the splanchnic nerves, both in intact animals and after bilateral adrenalectomy.

(b) Reversal of the vasopressor response to short periods of anoxia.

(c) Prevention of the splenic contraction largely responsible for the increase in circulating erythrocytes and mononuclear leukocytes normally induced in unanesthetized cats by a short struggle.

(d) Prevention or marked reduction of mydriasis and retraction of the nictitating membrane in response to electrical stimulation of the cervical sympathetic nerves or in response to injected epinephrine (even after denervation).

(e) Prevention of the pilomotor response to electrical stimulation of the lower abdominal sympathetic chains.

(f) Reversal of the nicotinic vasopressor response to large doses of choline esters in atropinized animals.

(g) Reversal of the epinephrine-induced contraction of the non-pregnant rabbit uterus both *in vivo* and *in vitro*.

6. Dibenamine provides protection against the lethal effects of several times the L.D.$_{100}$ of epinephrine.

7. The cardiac irregularities elicited by epinephrine in dogs anesthetized with cyclopropane are almost completely prevented by Dibenamine.

8. Preliminary experiments indicate that Dibenamine may be effective in lowering the blood pressure of rats with experimental renal hypertension.

9. Dibenamine does not prevent the inhibitory effects of epinephrine, such as relaxation of the intestine or the non-pregnant cat uterus. The rise in blood glucose and the hyperpnea induced by epinephrine are likewise unaffected.

10. The reversal of adrenergic vasopressor effects by Dibenamine is not altered by atropine, Benadryl or Pyribenzamine, or by the anesthetic employed.

11. Dibenamine apparently does not promote the *in vitro* or *in vivo* destruction of epinephrine.

These results are viewed as indicating that Dibenamine probably acts directly upon effector cells to prevent excitatory responses to epinephrine or sympathin E. The possible clinical usefulness of the adrenergic blocking action of Dibenamine is discussed.

REFERENCES

1. Dale, H. H.: J. Physiol., 34: 163, 1906.
2. Yonkman, F. F., Stilwell, D., and Jeremias, R.: This Journal, 81: 111, 1944.
3. Yonkman, F. F., Hays, H. W., Cameron, Anne, Pellett, Elizabeth, and Hansen, Nicoline: Fed. Proc., 5: 216, 1946.
4. Nickerson, M., and Goodman, L. S.: Proc. Am. Fed. Clin. Res., 2: 109, 1945.
5. Nickerson, M., and Goodman, L. S.: Fed. Proc., 5: 194, 1946.
6. Nickerson, M., Nomaguchi, G., and Goodman, L. S.: Fed. Proc., 5: 195, 1946.
7. Cannon, W. B., Newton, H. F., Bright, E. M., Menkin, V., and Moore, R. M.: Am. J. Physiol., 89: 84, 1929.
8. Grimson, K. S., Wilson, H., and Phemister, D. B.: Ann. Surg., 106: 801, 1937.
9. Hecht, H. H.: Personal communication. 1946.
10. Dale, H. H.: J. Physiol., 46: 291, 1913.
11. Cannon, W. B., and Lyman, H.: Am. J. Physiol., 31: 376, 1913.
12. Seager, L. D., Pruitt, M. C., Hippen, H. L., and Baldwin, R.: Fed. Proc., 4: 134, 1945.
13. Woodbury, L. A., and Nickerson, M. (To be published.)
14. Cannon, W. B., and Rosenblueth, A.: Am. J. Physiol., 104: 557, 1933.
15. Izquierdo, J. J., and Cannon, W. B.: Am. J. Physiol., 84: 545, 1928.
16. Menkin, V.: Am. J. Physiol., 85: 489, 1928.
17. Chess, Dorothy and Yonkman, F. F.: Proc. Soc. Exp. Biol. & Med., 61: 127, 1946.
18. Raab, W.: Exp. Med. and Surg., 1: 188, 1943.
19. Gernandt, B. E.: Acta Physiol. Scand., 11: Sup. 35, 1946.
20. Hamilton, W. F., Woodbury, R. A., and Vogt, E.: Am. J. Physiol., 125: 130, 1939.
21. Nickerson, M., Smith, S. M., and Goodman, L. S.: Fed. Proc., 5: 195, 1946.
22. Alpert, L. K., Alvino, A. S., and Grimson, K. S.: Proc. Soc. Exp. Biol. & Med., 37: 1, 1937.
23. Freeman, N. E., and Page, I. H.: Am. Heart J. 14: 405, 1937.
24. Jacobs, J., and Yonkman, F. F.: J. Lab. & Clin. Med., 29: 1217, 1944.
25. Grollman, A.: Proc. Soc. Exp. Biol. & Med., 57: 102, 1944.
26. Gilman, A., and Philips, F. S.: Science, 103: 409, 1946.
27. Goodman, L. S., Wintrobe, M. M., Dameshek, W., Goodman, M. J., Gilman, A., and McLennan, Margaret T.: J. A. M. A., 132: 126, 1946.
28. Jacobson, L. O., Spurr, C. L., Guzman Barron, E. S., Smith, T., Lushbaugh, C., and Dick, G. F.: J. A. M. A., 132: 263, 1946.
29. Morison, R. S., and Lissak, K.: Am. J. Physiol., 123: 404, 1938.
30. Bülbring, Edith, and Burn, J. H.: J. Physiol., 83: 483, 1935.
31. Mazur, A., and Bodansky, O.: J. Biol. Chem. 163: 261, 1946.
32. deTakats, G.: Surg., Gyn., & Obstet., 79: 359, 1944.
33. deTakats, G.: Arch. Neurol. & Psych., 50: 318, 1943.
34. White, J. C.: J. A. M. A., 124: 1030, 1944.
35. Nicholson, M. J.: Surg. Clinics No. Am., 24: 538, 1944.
36. Schafer, P. W.: Ann. Surg., 122: 1098, 1945.
37. Grimson, K. S.: Surgery, 19: 277, 1946.
38. Peet, M. M., and Isberg, E. M.: J. A. M. A., 130: 467, 1946.
39. Smithwick, R. H.: Arch. Surg., 49: 180, 1944.
40. Acheson, G. H., and Moe, G. K.: This Journal, 87: 220, 1946.
41. Berry, R. L., Campbell, K. N., Lyons, R. H., Moe, G. K., and Sutler, M. R.: Surgery, 20: 525, 1946.

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of the changes reported here. Blood samples were taken by cardiac puncture before and at intervals after injection of the thiourea compounds.

Glucose was measured by the method of Folin and Malmros (5) using a Somogyi (6) blood filtrate and glycogen according to Good et al. (7). Epinephrine was measured by the method of Bloor and Bullen (8) and lactic acid according to Barker and Summerson (9). A Klett-Summerson colorimeter was employed for the colorimetric measurements.

The procedure of Elliott et al. (10) was employed for measuring the oxidation of glucose by cell suspensions and anaerobic glycolysis was measured according to the procedure of Elliott and Henry (11). Whole cell suspensions were employed for the measurement of aerobic glycolysis in the same test system employed for measuring anaerobic glycolysis except that the gas phase consisted of 5% CO_2–95% O_2 and the rate of glycolysis was obtained from lactic acid measurements on trichloracetic acid filtrates one hour after the beginning of the experiment.

Adrenalectomy and hypophysectomy were performed under Nembutal anesthesia. Adrenalectomized animals were used six days after removal of the adrenal glands and hypophysectomized animals were used six weeks after removal of the pituitary glands. The cortin-treated adrenalectomized animals received 0.1 cc. (four rat units) of Lipo-Adrenal Cortex (Upjohn) per day intramuscularly.

Experimental. *Effect of ANTU and Related Compounds on the Blood Glucose of Rats.* Since the toxicity of thiourea to rats is quite variable and is influenced by such factors as age and diet (4) it is advisable to determine the toxicity of this compound to the particular group of animals employed. The toxicity of the four compounds employed in this investigation was measured prior to the examination of their effects on carbohydrate metabolism and the LD50 for rats was found to be 5 mgm./kgm. of ANTU, 5 mgm./kgm. of phenylthiourea, 500 mgm./kgm. of allylthiourea, and 2700 mgm./kgm. of thiourea.

In previous studies there was a correlation between the species susceptibility to ANTU and the dose required to produce hyperglycemia in dogs and rats. The differences in the toxicity of thiourea and the thiourea derivatives used in the present study made it possible to determine whether there was any correlation between the toxicity of the individual compounds to rats and the dose required to produce a disturbance in carbohydrate metabolism. For these experiments groups of 5 rats were given various doses of the compounds and blood glucose measurements were made at intervals during the first 10 hours after administration of the toxic compounds. Individual variations were small and in no case did they differ significantly from the average value for the group. All of the values shown in the accompanying figures are the average values for five animals.

The results of glucose measurements on ANTU-poisoned animals are shown in figure 1 where it may be seen that 10 mgm./kgm. (Curve A) and 100 mgm./kgm. (Curve B) of ANTU produced marked hyperglycemia, the time of onset and extent of the hyperglycemia being dependent upon the dose. While

STUDIES ON THE MECHANISM OF ACTION OF THIOUREA AND RELATED COMPOUNDS

III. The Effect of Acute Poisoning on Carbohydrate Metabolism[1]

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Received for publication December 2, 1946

Previous studies in this laboratory (1, 2) have shown that marked hyperglycemia and depletion of liver glycogen result from acute poisoning of rats and dogs by the new rodenticide, alpha-naphthylthiourea (ANTU). The extent of these disturbances in carbohydrate metabolism varied with the dose of the compound and the species susceptibility to the rodenticide.

The present investigation was undertaken for the purpose of finding (a) the cause of the hyperglycemia produced by ANTU, (b) methods of preventing and counteracting the effect, and (c) whether the disturbance in carbohydrate metabolism was an important factor in the lethal action of the rodenticide. The present communication contains the results of studies concerned with the effects of ANTU on the carbohydrate metabolism of rats and guinea pigs. With regard to the cause of the hyperglycemia the effect of ANTU on the carbohydrate metabolism of adrenal-demedullated and hypophysectomized rats was studied, and measurements of the effect of ANTU on glycolysis and on the oxidation of glucose were performed. The influence of insulin and ergotamine on the hyperglycemia and the toxicity of ANTU was examined.

Since similar changes in carbohydrate metabolism have not been demonstrated after acute poisoning by other mono-substituted derivatives of thiourea, as well as thiourea itself, it was of interest to measure the effects of thiourea and some thiourea derivatives on the carbohydrate metabolism of rats. Acute poisoning by these other compounds is similar to poisoning by ANTU in that they produce a delayed death with pulmonary edema and pleural effusion being the only prominent pathological changes (3, 4). Allylthiourea, phenylthiourea, and thiourea were employed for these studies since the large differences in toxicity of these three compounds to rats facilitated a comparison of the toxicity of the compounds with the doses required to produce hyperglycemia.

Methods. Adult male and female Sprague-Dawley rats and adult female guinea pigs were employed for these studies. Solutions of ANTU, phenylthiourea, and allylthiourea in anhydrous propylene glycol and aqueous solutions (25 per cent) of thiourea were injected intraperitoneally. The solutions were always prepared of such strength that less than 0.3% of the body weight was injected. This amount of propylene glycol was non-toxic and produced none

[1] This work was carried out under contract with the Medical Division of the Chemical Warfare Service.

2 (Curve C). However, a lethal dose (3000 mgm./kgm.) of thiourea produced a rise in blood glucose to 172 mgm.% in 3 hours (figure 2, Curve D). Thus, blood glucose measurements after acute poisoning of rats by thiourea clearly indicate that much larger quantities of the parent compound are necessary to produce disturbances in carbohydrate metabolism than were required in the case of the more toxic mono-substituted derivatives of thiourea.

The results of these experiments on blood glucose indicate that hyperglycemia results from acute poisoning of rats by ANTU, phenylthiourea, allylthiourea, and thiourea, and that there is a correlation between the toxicity of the individual compounds and the dose required to produce a disturbance in carbohydrate metabolism.

Effect of ANTU on the blood glucose of guinea pigs. It was of interest to find the amount of ANTU necessary to produce hyperglycemia in guinea pigs since

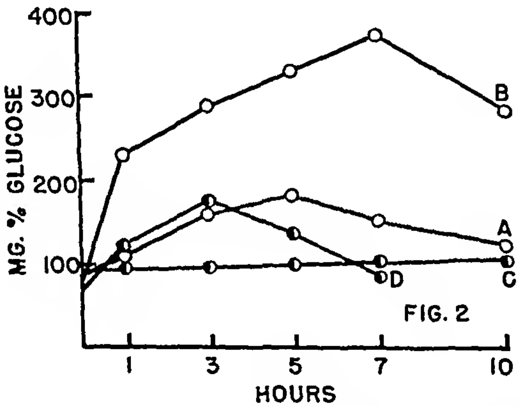

FIG. 2. THE EFFECT OF ALLYTHIOUREA AND THIOUREA ON THE BLOOD GLUCOSE OF RATS

Curve A, 200 mg./kg. allylthiourea; Curve B, 600 mg./kg. allylthiourea; Curve C, 600 mg./kg. thiourea; Curve D, 3000 mg./kg. thiourea

this species is resistant toward ANTU, the LD50 for our animals being 140 mgm./kgm. For this experiment adult female guinea pigs were given 50, 100, and 200 mgm./kgm. of ANTU and blood samples were taken at intervals for 10 hours. The results of these measurements are shown in figure 3 in which each curve represents the average values for 5 animals.

As shown in figure 3 the administration of 50 mgm./kgm. of ANTU to guinea pigs (Curve A) caused no appreciable increase in blood glucose while 100 mgm./kgm. (Curve B) produced an increase to 177 mgm.%, but the values returned to normal in 10 hours after administration of ANTU. This early return to normal did not occur in rats receiving this amount of the rodenticide. When a lethal dose of ANTU (200 mgm./kgm.) was given to guinea pigs (Curve C) the blood glucose increased to 188 mgm.% in 3 hours and then slowly decreased. The results of this experiment demonstrate that much higher doses of ANTU

none of the animals survived the doses employed in this experiment some occasionally survived for 24 hours and the blood glucose has always returned to normal by that time

Phenylthiourea is similar to ANTU in its toxicity to rats and the same quantities of phenylthiourea (10 and 100 mgm./kgm.) were therefore employed. The average blood glucose values are shown in figure 1 (Curves C and D) in which it may be seen that the blood glucose increased to values very near to those noted after similar doses of ANTU. Thus, acute poisoning by phenylthiourea also produces hyperglycemia in rats, the extent and the time of onset also being dependent upon the dose. The similarity in the effects of similar doses of ANTU and phenylthiourea on blood glucose is in agreement with the similar toxicity of these compounds to rats.

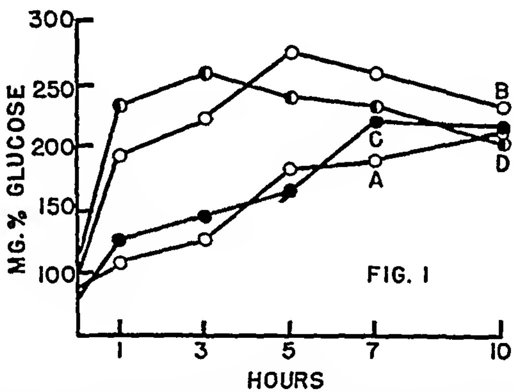

FIG. 1. THE EFFECT OF ANTU AND PHENYLTHIOUREA ON THE BLOOD GLUCOSE OF RATS

Curve A, 10 mg./kg. ANTU; Curve B, 100 mg./kg. ANTU; Curve C, 10 mg./kg. phenylthiourea; Curve D, 100 mg./kg. phenylthiourea.

Allylthiourea is considerably less toxic to rats than phenylthiourea or ANTU. For measurements of the effect of allylthiourea on the blood glucose of rats 200 and 600 mgm./kgm. of allylthiourea were employed. The results of blood glucose measurements are shown in figure 2 where it may be seen (Curve A) that a sublethal dose of allylthiourea (200 mgm./kgm.) produced an increase in glucose to 180 mgm.% in 5 hours and the value returned to normal in 10 hours. The hyperglycemia was neither as pronounced nor as persistent as was observed with much lower doses of the more toxic phenylthiourea and ANTU. After 600 mgm./kgm. of allylthiourea (Curve B) marked hyperglycemia occurred indicating that lethal doses of this compound also produce hyperglycemia in rats.

Thiourea was much less toxic to our rats than were any of the other compounds tested. In these experiments 600 mgm./kgm. of thiourea produced no change in blood glucose during a 10-hour sampling period as shown in figure

compound. The glycogen values for these animals and comparable controls are also shown in table 1. Each of the values listed is the average of measurements on five animals.

As shown in table 1 all of the compounds produced a decrease in liver glycogen of rats. The differences in the doses of each compound which were necessary to produce a decrease in liver glycogen are in agreement with the differences in the toxicity of these four compounds to rats.

Effect of insulin and ergotamine on the hyperglycemia produced by ANTU. In considering the possible cause of the hyperglycemia and depletion of liver glycogen produced by ANTU and related compounds attention was given to epinephrine since previous experiments (2) had indicated that insulin prolongs the time before the onset of hyperglycemia in dogs after ANTU. In the present

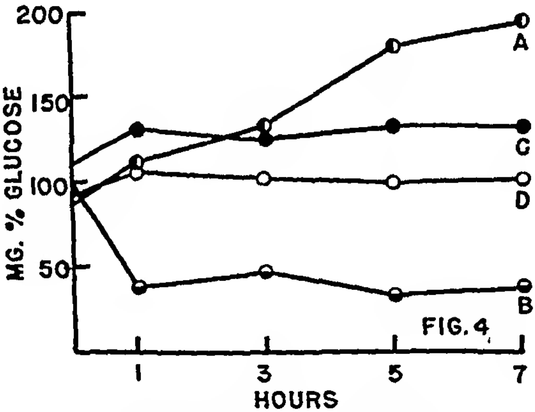

FIG. 4. THE EFFECT OF INSULIN, ERGOTAMINE, AND ADRENAL-DEMEDULLATION ON THE HYPERGLYCEMIA PRODUCED BY ANTU

Curve A, 10 mg./kg. ANTU; Curve B, 10 mg./kg. ANTU plus 3 units/kg. insulin; Curve C, 10 mg./kg. ANTU plus 10 mg./kg. ergotamine tartrate; Curve D, adrenal-demedullated rats given 10 mg./kg. ANTU.

experiments the effect of insulin and ergotamine on the hyperglycemia produced by 10 mgm./kgm. of ANTU in rats was tested since these two drugs antagonized the glycogenolytic effect of epinephrine. One group of five rats was given 10 mgm./kgm. of ANTU and served as the controls, while another group received the same amount of ANTU plus 3 units/kgm. of insulin subcutaneously. A third group of five rats received 10 mgm./kgm. of ANTU and 10 mgm./kgm. of ergotamine tartrate subcutaneously. The drugs were given within 5 minutes after the administration of ANTU. Blood glucose measurements were made at intervals for 7 hours after administration of ANTU and the average values for each group are shown in figure 4.

Curve A (figure 4) shows the progressive rise in blood glucose to 195 mgm.% in 7 hours produced by 10 mgm./kgm. of ANTU. Insulin (Curve B) and er-

are necessary to produce hyperglycemia in guinea pigs than in rats, in agreement with the greater resistance of guinea pigs toward the rodenticide.

Effect of thiourea and related compounds on liver glycogen. The experiments presented above indicate that acute poisoning by mono-substituted derivatives of thiourea, and thiourea itself, produce similar changes in carhohydrate metabolism. Previous experiments (1, 2) had shown that ANTU produces a depletion of liver glycogen. It, therefore, seemed likely that other thiourea compounds would also cause a reduction in liver glycogen and this was found to he the case.

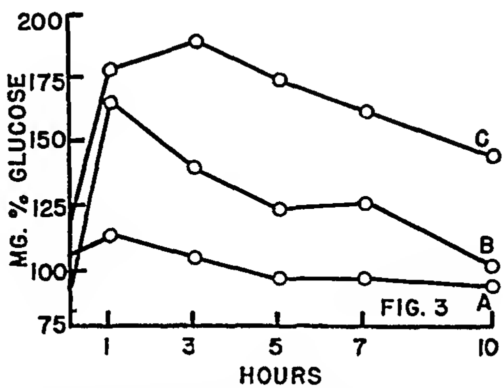

Fig. 3. The Effect of ANTU on the Blood Glucose of Guinea Pigs
Curve A, 50 mg./kg.; Curve B, 100 mg./kg.; Curve C, 200 mg./kg.

TABLE 1

Effect of thiourea and related compounds on liver glycogen of rats

Compound	Males fasted 19 hours		Females fasted 6 hours	
	mgm./kgm.	% Glycogen	mgm./kgm.	% Glycogen
Control		0 50		1.29
ANTU..	10	0.03	10	0.76
Phenylthiourea.	10	0.11	10	0.35
Allythiourea	1000	0.28	1000	0.03
Thiourea. ..	3000	0 23	2500	0 55

For glycogen measurements the control animals were fasted for 19 hours and the poisoned animals were fasted for 13 hours before injection of the toxic compound and for 6 hours after poisoning and were then sacrificed for glycogen determinations. The liver glycogen values after administration of lethal doses of thiourea, allylthiourea, phenylthiourea, and ANTU are shown in table 1 together with the average values for the control animals. Other measurements were carried out on female rats fasted for 6 hours after injection of the toxic

after ANTU for the chemical measurement of epinephrine. Although the glucose rose to 186 mgm.% in 7 hours after ANTU no epinephrine could be detected in the blood. Determination of the rate of disappearance of injected epinephrine showed that there was no difference between normal and ANTU-poisoned rats. When 65 micrograms of epinephrine was injected into the tail veins of normal rats and rats which had received 10 mgm./kgm. of ANTU 3 hours previously the average recovery was 37% for the control animals and 32% for the ANTU-poisoned group in 1 minute after injection of epinephrine, there being no significant difference in the rate of disappearance of injected epinephrine from the blood of normal and ANTU-poisoned rats.

Effect of ANTU and phenylthiourea on glycolysis and the oxidation of glucose. The absence of the disturbance in carbohydrate metabolism in ANTU-poisoned rats after removal of the adrenal medulla and the antagonistic action of insulin and ergotamine indicated that increased glycogenolysis rather than inhibition of the utilization of carbohydrate was the cause of the hyperglycemia produced by ANTU. However, to insure that ANTU and phenylthiourea had no direct inhibitory effect on glycolysis or on the oxidation of glucose the effect of these substances on anaerobic and aerobic glycolysis, and on the oxidation of glucose was studied *in vivo* and *in vitro.*

Whereas the rate of anaerobic glycolysis ($Q\ N_2/CO_2$) for normal rat brain was 8.8 the value for brain from rats sacrificed 4 hours after ANTU was 8.2, there being no significant difference in the rate of anaerobic glycolysis of brain from normal and ANTU-poisoned animals. Similarly, there was no effect on anaerobic glycolysis of lung tissue. Neither ANTU (1.25×10^{-5} M) nor phenylthiourea (5×10^{-3} M) had any inhibitory effect *in vitro* on anaerobic glycolysis of rat brain or lung tissue. Aerobic glycolysis of brain and lung tissue was not inhibited *in vivo* nor *in vitro* by ANTU or phenylthiourea.

The oxidation of glucose by brain tissue was not inhibited by ANTU. Whereas the average Q_{O_2} values for 4 normal rats was 3.9 that for 4 rats sacrificed 4 hours after administration of 10 mgm./kgm. of ANTU was 3.8. There was no significant decrease in the rate of oxidation of glucose by lung tissue. Phenylthiourea (3×10^{-3} M) and ANTU (2.5×10^{-5}M) had no effect on the oxidation of glucose by brain and lung tissues. The results of these experiments indicate that neither ANTU nor phenylthiourea have any direct effect on glycolysis or the oxidation of glucose.

Discussion. The physiological changes which result from acute poisoning by ANTU and related compounds are of importance because of the possible wide use of ANTU as a rodenticide and of certain other thiourea derivatives (12) in the treatment of hyperthyroidism. While the response of animals to lethal doses of many thiourea compounds is similar, as evidenced by the intense pulmonary edema and pleural effusion (3, 4), no evidence of similar chemical changes has been presented previously.

The studies presented here indicate that thiourea, allylthiourea, phenylthiourea, and ANTU all produce hyperglycemia in rats. The wide difference in toxicity of these compounds facilitate a comparison of the toxicity of individual com-

gotamine (Curve C) effectively antagonized the hyperglycemic effect of ANTU. The antagonistic action of both of these substances gave strong indications that the hyperglycemia produced by ANTU was due to the glycogenolytic action of epinephrine.

Effect of adrenalectomy and hypophysectomy on the hyperglycemia produced by ANTU. In order to obtain further information concerning the cause of the disturbance in carbohydrate metabolism after acute poisoning by ANTU experiments were carried out on adrenalectomized and adrenal-demedullated rats. For these experiments a group of five adrenalectomized cortin-treated rats were given 10 mgm./kgm. of ANTU and blood glucose was followed for 10 hours. During this period there was no increase in blood glucose throughout the sampling period. Liver glycogen measurements on a group of five adrenalectomized cortin-treated rats gave an average value of 1.3% indicating that the liver glycogen was at a normal level at the time of administration of ANTU, and failure to obtain hyperglycemia in adrenalectomized animals after ANTU was, therefore, not due to inadequate liver glycogen at the time of administration of ANTU.

In order to eliminate the possible influence of injected cortin on carbohydrate metabolism after ANTU other experiments were carried out on adrenal-demedullated rats. Six days after removal of the adrenal medulla liver glycogen was determined on a group of five of the animals and the average value for the group was 0.77%. Another group of five adrenal-demedullated animals was given 10 mgm./kgm. of ANTU and blood glucose was measured for 7 hours after administration of the rodenticide. Curve D (figure 4) shows that there was no hyperglycemia following the administration of ANTU to adrenal-demedullated animals, thus indicating that the adrenal medulla is involved in the production of the hyperglycemia and depletion of liver glycogen after acute poisoning by ANTU.

Another group of five adrenal-demedullated rats was given 10 mgm./kgm. of ANTU and the survival times observed to determine the effect of adrenal-demedullation on the survival time of ANTU-poisoned rats. All of the animals exhibited pulmonary edema and pleural effusion and died between 24 and 48 hours after the administration of ANTU. Thus, while the survival time was a few hours greater than for normal animals after 10 mgm./kgm. of ANTU, prevention of hyperglycemia by demedullation did not protect the animals from the lethal effects of 10 mgm./kgm. of ANTU.

The effect of 10 mgm./kgm. of ANTU on hypophysectomized rats was also measured. Hypophysectomy had no effect on the time of appearance, the extent, nor the persistence of the hyperglycemia, and it did not influence the survival time nor the extent of the damage to the lungs.

Epinephrine content of the blood of ANTU-poisoned rats. Because of the above evidence that the adrenal medulla was involved in the hyperglycemic effect of ANTU it was of interest to measure the epinephrine content of the blood of ANTU-poisoned rats in order to ascertain whether ANTU caused an increased liberation of epinephrine. Accordingly, rats were given 20 mgm./kgm. of ANTU intraperitoneally and blood samples were taken at 1, 3, 5, and 7 hours

ment with the previous findings of Bernheim and Bernheim (14) who demonstrated that phenylthiourea had no inhibitory action on this oxidative process. The absence of an inhibitory effect of these substances on glycolysis and the oxidation of glucose is in agreement with the results obtained with adrenal-demedullated rats in which the glucose remained at normal values.

SUMMARY

1. Acute poisoning by ANTU, phenylthiourea, allylthiourea, and thiourea results in hyperglycemia in rats, the time of appearance, extent, and duration of hyperglycemia being dependent upon the dose of the compounds administered and upon the toxicity of the individual compounds to rats. Higher quantities of ANTU were necessary to produce hyperglycemia in guinea pigs than in rats in agreement with the difference in toxicity of the rodenticide to those two species.

2. Lethal doses of ANTU, phenylthiourea, allylthiourea, and thiourea produced a marked drop in liver glycogen in rats.

3. Both insulin and ergotamine antagonized the hyperglycemic effect of 10 mgm./kgm. of ANTU, and adrenal-demedullation prevented the hyperglycemia, but the survival time of rats was not increased by insulin, ergotamine, or adrenal-demedullation.

4. Hypophysectomy did not prevent the hyperglycemia or increase the survival time of rats after 10 mgm./kgm. of ANTU.

5. There was no detectable increase in the epinephrine content of blood from ANTU-poisoned rats.

6. Neither ANTU nor phenylthiourea inhibited glycolysis or the oxidation by glucose by brain or lung tissue.

Acknowledgement. The authors are indebted to Dr. E. M. K. Geiling, Professor of Pharmacology, University of Chicago, and to Dr. J. O. Hutchens, Director, University of Chicago Toxicity Laboratory for helpful suggestions during the course of this investigation, and to the Upjohn Laboratories for the Adrenal Cortical Extract.

BIBLIOGRAPHY

1. DuBois, K. P., Holm, L. W., and Doyle, W. L.: Proc. Soc. Exp. Biol. and Med., 61: 102, 1946.
2. DuBois, K. P., Holm, L. W., and Doyle, W. L.: This Journal, 87: 53, 1946.
3. Richter, C. P.: J. A. M. A., 129: 927, 1945.
4. Dieke, S. H., and Richter, C. P.: This Journal, 83: 195, 1945.
5. Folin, O., and Malmros, H.: J. Biol. Chem., 83: 115, 1929.
6. Somogyi, M.: J. Biol. Chem., 86: 655, 1930.
7. Good, C. A., Kramer, H., and Somogyi, M.: J. Biol. Chem., 100: 484, 1933.
8. Bloor, W. R., and Bullen, S. S.: J. Biol. Chem., 138: 727, 1941.
9. Barker, S. B., and Summerson, W. H.: J. Biol. Chem., 138: 535, 1941.
10. Elliott, K. A. C., Scott, D. B. M., and Libet, B.: J. Biol. Chem., 146: 251, 1942.
11. Elliott, K. A. C., and Henry, M.: J. Biol. Chem., 163: 361, 1946.
12. Astwood, E. B.: This Journal, 78: 79, 1943.
13. Murray, H. C., and Morgan, A. F.: J. Biol. Chem., 163: 401, 1946.
14. [illegible] heim, M. L. C.: J. Biol. Chem., 145: 213, 1942.

pounds with the doses required to produce hyperglycemia in rats, in which it was noted that quantities near of above the lethal dose of each compound were necessary in order to produce the hyperglycemic response. Thus, it is possible to predict whether animals have received a lethal dose of the compounds by measuring blood glucose at intervals during the first 10 hours after injection of the compounds. Further evidence that the hyperglycemia is associated with lethal doses of ANTU was obtained in experiments with guinea pigs in which it was found that much higher doses of ANTU were required to produce hyperglycemia in this resistant species than in rats. That the disturbance in carbohydrate metabolism did not always persist until death was seen in cases where animals survived for 24 hours since the blood glucose values for these animals had returned to normal.

The depletion of liver glycogen which resulted from acute poisoning by all of the compounds can be explained by increased glycogenolysis. The antagonistic action of insulin and ergotamine toward the hyperglycemia produced by ANTU together with the absence of the hyperglycemic effect in ANTU-poisoned adrenal-demedullated rats give strong indications that epinephrine is involved in the hyperglycemic effect of ANTU and related compounds. Since the experiments presented here indicate that ANTU does not cause an increased liberation of epinephrine it seems more likely that the glycogenolytic effect might be due to a failure of tissues from ANTU-poisoned animals to inactivate epinephrine at a normal rate. Another factor which remains to be investigated is the possible influence of certain cellular substances on the glycogenolytic action of epinephrine as has been suggested recently by Murray and Morgan (13) in experiments on the carbohydrate metabolism of ascorbic acid deficient guinea pigs. While much attention has been given to the effect of thiourea derivatives on the thyroid gland the present study provides the first evidence that the adrenals are affected by these compounds.

While hyperglycemia results from poisoning by many chemical compounds in most cases the significance of this disturbance as a contributing factor in the lethal action of the particular compound has not been determined. In the present studies it has been possible to prevent the disturbance in carbohydrate metabolism without appreciably affecting the resistance of the animals toward ANTU, thus indicating that the effect of ANTU on carbohydrate metabolism is not the major factor in its acute toxic effects on animals. Although the hyperglycemia was prevented by insulin, ergotamine, and adrenal-demedullation these treatments did not prevent the pulmonary edema and pleural effusion which appear to be the direct cause of death. It is interesting that the hyperglycemia is so closely associated with lethal doses of the compounds but does not contribute appreciably to their lethal action. Further investigations may reveal, however, that there is some relationship between the factors responsible for the hyperglycemia and those responsible for the damage to the capillaries.

The failure of ANTU and phenylthiourea to inhibit glycolysis and the oxidation of glucose indicate that these substances do not inhibit the utilization of carbohydrate. The absence of an effect on the oxidation of glucose is in agree-

tion of BAL was the 10 per cent solution in peanut oil and benzyl benzoate as prepared for human use (4) (5), but diluted with U.S.P. peanut oil to form a 1 to 2 per cent solution.

Animals alive and well thirty days after the injection, with no loss in body weight, were considered to have been protected by BAL. With 3 of the compounds, the control animals, not treated with BAL, regularly died in one to three days; with the fourth compound, the p-CH_3 phenylstibonic acid, death was significantly delayed, and control animals died in one to 12 days.

Effect of BAL on antimony excretion. To determine the effect of BAL on the urinary excretion of antimony, animals were injected intravenously with each of the compounds at the LD_{50} level. After 24 hours in a metabolism cage, the bladder was catheterized and irrigated, and the specimen pooled with that collected in the cage. The animal was then tied down, and two successive

TABLE 1

The acute toxicity in rabbits of Anthiomaline, tartar emetic, Fuadin and p-CH_3 phenylstibonic acid on single intravenous injection

COMPOUND	DOSE	NO. OF RABBITS	DIED	SURVIVED	LD_{50},	LD_{99},
	mg./kg.				mg./kg.	mg./kg.
Anthiomaline	15	3	3	0		
	12.5	3	3	0	8.5	12
	10	7	6	1		
	7.5	6	2	4		
Tartar emetic	15	7	6	1		
	12.5	5	3	2	12	15
	10	6	1	5		
	7.5	3	0	3		

COMPOUND	DOSE	NO. OF RABBITS	DIED	SURVIVED	LD_{50},	LD_{99},
	mg./kg.				mg./kg.	mg./kg.
Fuadin	150	7	7	0		
	100	7	4	3	90±	150±
	75	3	0	3		
p-methyl-phenyl stibonic acid	12.5	10	10	0		
	10	6	5	1		
	8	6	5	1	7±	10±
	6	5	1	4		
	4	5	2	3		
	3	3	0	3		

2-hourly urine specimens were collected by catheterization and irrigation prior to the administration of BAL (single intramuscular injection at 20 mg./kg.). Two similar specimens were collected after the injection of the BAL; and the animals were then placed in a metabolism cage for the collection of the following 24-hour urine specimen.

Two animals were tested with each compound; and because of deaths occurring in 24 hours at the LD_{50} dosage, it was necessary to inject a larger number of animals in order to obtain at least two survivors.

With tartar emetic, one rabbit was similarly treated with BAL 72 hours after the injection of the antimonial.

The antimony content of the urine specimens was determined by the Maren (7)-Freedman (8) modification of the rhodamine B method.

Experimental Results. A. *The effect of BAL on the survival of rabbits*

THE PROTECTIVE ACTION OF BAL IN EXPERIMENTAL ANTIMONY POISONING

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Received for publication December 7, 1946

The discovery of 2,3-dimercaptopropanol ("BAL") by Peters, Stocken, Thompson and their associates has been described in several recent reviews (1) (2). These workers demonstrated its pronounced affinity for arsenicals, its ability to remove arsenic from combination with tissues, and its detoxifying action when applied to the skin of animals poisoned with arsenical blister gases. Previous communications from this laboratory have described the development of a stable solution of BAL in peanut oil and benzyl benzoate suitable for intramuscular injection (3), the efficacy of that solution in the systemic treatment of experimental arsenic poisoning with mapharsen, lewisite, and phenyl arsenoxide (3), and the results in the treatment of 227 human cases of arsenic poisoning (encephalitis, dermatitis, blood dyscrasias, jaundice and fever) occurring as complications of arsenotherapy (4) (5).

It was a natural extension of these studies to determine the efficacy of BAL in other types of heavy metal poisoning, involving elements which might reasonably be expected to form stable cyclic linkages with BAL, resembling those formed by arsenic. The present paper will describe such studies with a series of organic antimonials, some of which have been used in the treatment of tropical diseases. In a similar study carried out independently, Braun, Lusky and Calvery (6) have shown that the lethal dose (LD_{50}) of antimony potassium tartrate, "Fuadin" and "Neostam" was increased more than 50 per cent by the administration of BAL in relatively large doses (30, 15, 15 and 18 mg./kg.) 1, 6, 24 and 48 hours after the antimonial.

Methods and Materials. The antimonials used in the present study were "Anthiomaline" (antimony lithium thiomalate), "Fuadin" (sodium antimony bis-catechol-2,4-disulfonate), tartar emetic (antimony potassium tartrate), and p-methylphenylstibonic acid. The "Anthiomaline" and "Fuadin" were generously supplied by Merck and Company and the Winthrop Chemical Company, respectively. The last-named compound was prepared in this laboratory (12).

Effect of BAL on survival. With each of these 4 compounds, the lethal dose on a single intravenous injection was first determined. A series of rabbits were then given a dose well in excess of the LD_{95} value, and treatment will BAL was begun 2 hours later, at dosages per injection varying from 20 down to 5 mg. per kg. Treatment with BAL was repeated every 4 hours to a total of 4 doses on the first day, tapering off thereafter as indicated in the Tables. The stock prepara-

administration of BAL had a regular and definite protective action. With all four compounds, dosages of BAL on the order of 10 to 15 mg. per kg. saved approximately half the animals. Although it is not indicated in table 2, death was significantly delayed in many of the animals which ultimately died. Some of these might perhaps have been saved had intensive treatment with BAL been continued for a period of e.g. 7 days.

B. *The effect on BAL on the urinary excretion of antimony.* In the case of arsenicals, it had previously been shown that the protective action of BAL was

TABLE 3

The effect of BAL on the urinary excretion of Anthiomaline

Rabbit A-5523 (3.6 kg.) was injected intravenously with 0.52 cc. of a 6% solution of Anthiomaline (16.7% antimony). The dosage of 8.6 mg./kg. was the LD_{50} level of this compound, and represented a total of 5150 micrograms of antimony. Urine was obtained by bladder catheterization and irrigation at the times indicated. BAL was given intramuscularly 28 hours after the antimonial, as a single injection at 20 mg./kg. (3.6 cc. of a 2% solution in peanut oil).

TIMES OF URINE COLLECTION AFTER INJECTION	VOL. OF COMBINED URINE AND WASHINGS	ANTIMONY		TOTAL ANTIMONY IN SPECIMEN	% OF ANTIMONY INJECTED	CUMULATIVE TOTALS EXCRETED IN URINE		HOURLY EXCRETION OF ANTIMONY	
						Micrograms	% of amt. injected	Micrograms	% of amt. injected
hours	*cc.*	*micrograms/cc.*		*micrograms*					
0–2	21.1	40.7 39.6	40.2	848.0	16.5	848.2	16.5	424.1	8.3
2–24	135	10.8 9.9	10.4	1404.0	27.3	2252.2	43.8	63.8	1.3
24–26	40.6	2.2 2.0	2.1	85.3	1.7	2337.5	45.5	42.7	0.9
26–28	17.8	4.0 4.6	4.3	76.5	1.5	2414.0	47.0	38.3	0.8
BAL 28–30	14.4	18.5 18.3	18.4	265.0	5.1	2679.0	52.1	132.5	2.6
30–32	21.4	6.7 6.3	6.5	139.1	2.7	2818.1	54.8	69.6	1.4
32–56	148	4.7 4.1	4.4	651.2	12.6	3469.3	67.4	27.1	0.5

associated with an increased urinary excretion of arsenic (3–5)(9)(10). With highly toxic compounds such as phenyl arsenoxide or lewisite, which are firmly bound by the tissues and which are excreted only slowly (11), the rate of urinary excretion was increased as much as a hundred-fold by the administration of BAL (3). Less toxic compounds, such as mapharsen, are normally excreted far more rapidly. This rapid excretion tends to mask the increased excretion caused by BAL; but even with mapharsen, in both rabbits (3) and man (4) (5) (9), the administration of BAL was followed by a significantly increased urinary excretion of arsenic.

receiving otherwise lethal doses of antimonial. Preliminary experiments to determine the acute toxicity for rabbits of the 4 compounds here studied are summarized in table 1. The LD_{95} values of Fuadin, tartar emetic, Anthiomaline,

TABLE 2

The protective action of BAL in rabbits poisoned with LD_{95} doses of antimonials

Animals were injected intravenously with a single dose of antimonial at 1 to 1.5 times the LD_{95} level. Intramuscular treatment with BAL in peanut oil and benzyl benzoate was begun 2 hours later at the dosages indicated in the table, and repeated every 4 hours to a total of 4 doses in the first 24 hours. Most of the animals received two additional injections of BAL at the same dosage level in the second 24 hours; the others, indicated with an asterisk (*) in the table, instead received single daily injections on the 2nd, 3rd and 4th day.

Antimonial injected: Compound	Antimonial injected: Mg./kg.	Dosage of BAL per injection	No. of animals	Survived	Died	Dose of BAL per injection which saved approximately half of animals
		mg./kg.				
		0*		0		
		2.5	6	0	6	
		5*	5	0	5	
Anthiomaline	12	7.5*	6	0	6	10–15 mg./kg.
		10	8	4	4	
		15	8	4	4	
		20	8	1	7	
		0		0		
		5	6	2	4	
Fuadin	150	10	7	2	5	15
		15	7	3	4	
		20	7	6	1	
		0		0		
		2.5*	8	0	8	
	17	5*	8	1	7	
		7.5*	8	3	5	
Tartar emetic		10*	6	2	4	15
		10	7	2	5	
	15	15	7	3	4	
		20	7	3	4	
		0		0		
		7.5	7	2	5	
p-CH_3-phenyl stibonic acid	12.5	10	9	5	4	10—15
		15	7	1	6	
		20	8	3	5	

and p-methylphenylstibonic acids were, respectively, 150, 15, 12 and 10 mg./kg. Animals were then given doses of antimonials significantly in excess of that LD_{95} level, and treatment with BAL, in varying dosage, was begun 2 hours later.

All the control animals, receiving no BAL, died. As shown in table 2, the

A single experiment in which the BAL was given 72 hours after an LD_{50} dose of tartar emetic is summarized in table 5. Despite the long interval, and despite the fact that by far the greater portion of the antimony injected had already

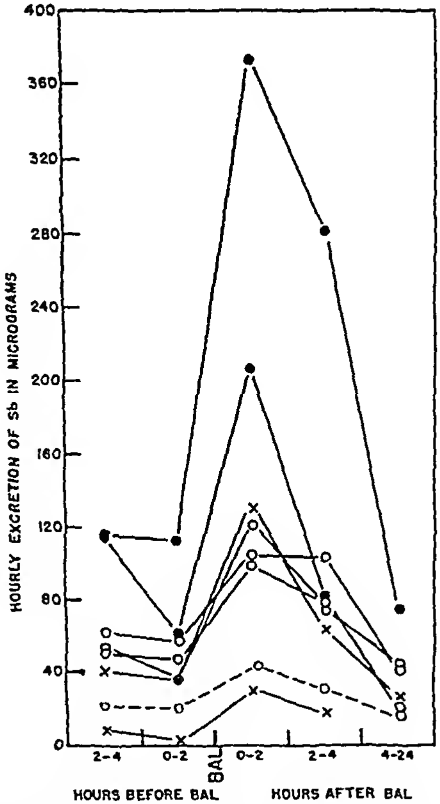

FIG. 1. THE EFFECT OF BAL ON THE URINARY EXCRETION OF ANTIMONY
(After data of tables 4 and 5)
●—● Fuadin
x—x Anthiomaline
O—O Tartar emetic

All rabbits were injected intravenously with LD_{50} dosages of the various antimonials (cf table 4) BAL was injected intramuscularly 28 hours later at a dosage of 20 mg /kg, given as a single injection of a solution in peanut oil and benzyl benzoate In one experiment (curve O—O in the figure) BAL was given 72 hours after the antimonial Urine samples were collected by catheterization at the time period indicated in the figure The abscissae in the figure are the hourly urinary excretion of antimony in micrograms

been excreted, BAL caused a significant increase in the rate of urinary antimony excretion For the first two hours, the rate of excretion increased from the pre-BAL level of 21 micrograms of antimony per hour to 45. It averaged 32

There was reason to believe that, as with arsenicals, the protective action of BAL in experimental antimony poisoning reflected its ability to remove antimony from an otherwise fatal combination with vital components of the body tissues. However, since three of the antimonials here used had toxicities of the same order of magnitude as that of mapharsen (LD_{50} of 13 mg./kg.), while one was even less toxic, there was the possibility that the increased urinary excretion of relatively small amounts of antimony liberated from the tissues by BAL might be masked by the rapid urinary excretion occurring independently of BAL.

One of 2 experiments with Anthiomaline is given in detail in table 3. Similar experiments with all 4 compounds are summarized in table 4, and in figs. 1 and 2. The hourly urinary excretion of antimony before and after the injection of BAL is plotted in fig. 1 in absolute terms (micrograms per hour), and in fig. 2 as the percentage of the immediate pre-BAL level.

TABLE 4

Effect of BAL on rate of antimony excretion of Fuadin, Anthiomaline, tartar emetic and p-methylphenyl-stibonic acid

COMPOUND	MG./KG.	ANIMAL NO.	TOTAL ANTIMONY INJECTED, MICROGRAMS	HOURLY EXCRETION OF ANTIMONY IN MICROGRAMS					HOURLY EXCRETION OF ANTIMONY IN % OF AMOUNT INJECTED					INCREASED EXCRETION CAUSED BY BAL (RATES REFERRED TO PRE-BAL LEVEL AS 100)		
				Pre-BAL			Post-BAL		Pre-BAL			Post-BAL				
				28-4	4-2	2-0	0-2	2-4	28-4	4-2	2-0	0-2	2-4	1st 2 hrs.	2nd 2 hrs.	24 hrs. thereafter
Fuadin	90	A-5396	52,500	877.8	117.4	114.6	376.1	282.6	1.7	0.2	0.2	0.7	0.6	328	246	68
		A-5761	32,600	813.3	116.6	60.8	208.5	83.2	2.5	0.4	0.2	0.7	0.3	344	137	—
Anthiomaline	8.6	A-5523	5,150	93.9	42.7	38.3	132.5	69.6	1.8	0.9	0.8	2.6	1.4	346	183	71
		A-5746	3,726	17 2	8.4	3.7	31.6	19.2	0.5	0.2	0.1	0.9	0.5	854	519	—
Tartar emetic	12	A-5515	10,500	193.3	52.0	49.0	101.7	76.5	2.3	0.5	0.5	1.0	0.8	208	156	86
		A-5398	14,400	195.1	63.9	58.6	96.2	95.2	1.4	0.4	0.4	0.7	0.7	164	162	78
		A-5623	9,400	228.8	54.8	36.4	122.6	80.5	2.4	0.6	0.4	1.3	0.9	337	221	63
p-Methylphenyl-stibonic acid	6.7	A-5566	9,861	151 3	1.6	2.5	3.7	6.6	1.5	0.0	0.1	0.1	0.1	148	264	144
		A-5629	9,445	132.0	3.2	2.1	2.5	2.5	1.4	0.1	0.0	0.1	0.1	119	119	10

It is clear that with tartar emetic, Fuadin and Anthiomaline, all injected at the LD_{50} level, a single injection of BAL at 20 mg. per kg., given 28 hours after the antimonial, was followed by a significant increase in the urinary excretion of antimony. For the first 2 hours after the injection of BAL, the rate of excretion was 2 to 8 times greater than the pre-BAL rate, averaging 3 times greater; in the second 2 hours it was 1 to 5 times greater, averaging 2; and in the following 24 hours, it was at the pre-BAL level or less. Only with the p-CH_3 phenylstibonic acid did BAL fail to cause an increased urinary excretion, this despite the fact that it did save a significant proportion of animals injected at the LD_{95} level. It is particularly to be noted in figs. 1 and 2 that in every animal studied, the rate of antimony excretion was falling prior to the administration of BAL. The 2- to 8-fold increase observed after its administration is clearly to be related to the injection of BAL, and is not fortuitous.

margin of safety consistent with a maintained action of BAL, may therefore be equally applicable in the treatment of antimony poisoning.

TABLE 5

The effect of BAL on the urinary antimony excretion seventy-two hours after the administration of tartar emetic

Rabbit A-5673 (2.72 kg.) was injected intravenously with 3.25 cc. of a 1% solution of tartar emetic (36.5% antimony) at the LD_{50} level (12 mg./kg., representing a total of 11,800 micrograms of antimony). BAL was administered intramuscularly 72 hours after the antimonial, as a single injection at 20 mg./kg. (2.72 cc. of a 2% solution in peanut oil). Three successive 2-hour urine specimens were obtained by bladder catheterization and irrigation before and after the BAL injection.

HOURS OF URINE COLLECTION, RELATIVE TO INJECTION OF BAL	VOL. OF COMBINED URINE AND WASHINGS	ANTIMONY	TOTAL ANTIMONY IN SPECIMEN	% OF ANTIMONY INJECTED	CUMULATIVE TOTALS EXCRETED IN URINE		HOURLY EXCRETION OF ANTIMONY	
					Micrograms	% of amt. injected	Micrograms	% of amt. injected
	cc.	*micrograms/cc.*	*micrograms*					
−6 – −4	33.8	1.2	40.6	0.3	40.6	0.3	20.3	0.2
−4 – −2	25.8	1.8	46.4	0.4	87.0	0.7	23.2	0.2
−2 – 0	23.6	1.8	42.5	0.4	129.5	1.1	21.3	0.2
BAL								
0 – 2	28.1	3.2	90.0	0.8	219.5	1.9	45.0	0.4
2 – 4	21.6	3.0	64.8	0.6	284.3	2.5	32.4	0.3
4 – 6	19.3	1.8	34.7	0.3	319.0	2.8	17.4	0.2

SUMMARY

1. On single intravenous injection, the lethal (LD_{95}) levels of Fuadin, tartar emetic, Anthiomaline and p-methylphenylstibonic acid were found to be 150, 15, 12 and 10 mg./kg., respectively.

2. BAL had a definite protective action in rabbits given otherwise lethal doses of these antimonials. With all four compounds, injections of BAL at 10 to 15 mg./kg. administered 4 times at 4-hour intervals saved approximately half the animals.

3. As has been previously shown to be the case with arsenicals, the protective action of BAL in experimental antimony poisoning was, in 3 of the 4 compounds tested, associated with and probably due to a significantly increased urinary excretion of antimony. The hourly rate of excretion increased 2- to 8-fold in rabbits given a single injection of BAL at 20 mg./kg., 28 hours after the injection of the antimonial at the LD_{50} level. This favorable effect on excretion lasted for 2 to 4 hours, indicating the need for repeated injections at approximately 4-hour intervals when BAL is used therapeutically in cases of antimony poisoning.

BIBLIOGRAPHY

1. Peters, R. A., Stocken, L. A., and Thompson, R. H. S.: Nature, 156: 616, 1945.
2. Waters, L. L., and Stock, C. C.: Science, 102: 601, 1945.

in the next two-hour period, and had reverted to the "normal" level of 17 micrograms per hour in the third 2-hour period. This experiment is indicated by the dotted circles in figs. 1 and 2.

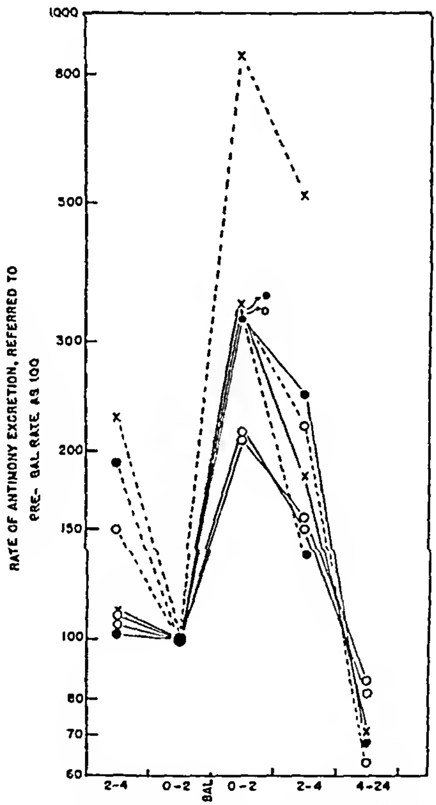

FIG. 2. THE EFFECT OF BAL ON THE URINARY EXCRETION OF ANTIMONY

(After data of tables 4 and 5)

The experimental data have here been plotted on a percentage basis, referred to the 2-hour period immediately preceding the injection of BAL as 100. The symbols have the same significance as in figure 1.

With the arsenicals, it had been observed in both rabbits (3) and normal human subjects (10) that the favorable effect of BAL on arsenic excretion lasted for only 2 to 4 hours, reflecting both the rapid excretion of BAL and its destruction in vivo. It is evident that this is true also of the antimonials here studied. The four-hourly schedule developed for the administration of BAL in the treatment of arsenic poisoning in man (4) (5), designed to provide the maximum

THE TOXICITY AND ANALGETIC POTENCY OF SALICYLAMIDE AND CERTAIN OF ITS DERIVATIVES AS COMPARED WITH ESTABLISHED ANALGETIC-ANTIPYRETIC DRUGS[1]

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Received for publication October 14, 1946

The search for new, non-opiate analgetics which has been in progress in recent years seems to have by passed the salicyl compounds. The reasons for this are not clear, but low water-solubility may be a factor. Salicylamide has been studied in various aspects by various authors (quoted by Houben (1)), but there appear to be no studies of its analgetic action nor are there studies of its toxicity as compared with other analgetics. It has been reported that salicylamide is hydrolyzed by various enzymes and tissue fluids (2, 3), but these studies cannot be relied upon since the tests used are not adequately specific to distinguish between salicylamide and certain other salicylates.

Since salicylamide has been shown to be absorbed from the intestinal tract (4, 5) we reinvestigated this compound. We have also studied the two possible acetyl derivatives of salicylamide and two of its many possible halogen derivatives. These have been compared with sodium salicylate, acetylsalicylic acid, antipyrine, aminopyrine, acetanilid and acetophenetidin in respect to toxicity and analgetic potency.

METHODS. As indicated above most of the compounds used in this study are poorly soluble in water. Thus parenteral administration would be possible only with aqueous solutions of unphysiologic acidity or alkalinity or with solutions in other solvents. Therefore we have used oral administration exclusively. Aqueous suspensions of the drugs (usually in 5 or 10 per cent concentration) proved to be satisfactorily stable when gum tragacanth was included in 1.0 or 1.5 per cent concentration. These suspensions were administered from a hypodermic syringe of suitable size through a stomach tube. For mice and rats the stomach tubes were made from hypodermic needles (18 and 14 G respectively) by grinding off the bevel. For rabbits a size 16F rubber catheter served as stomach tube.

Deaths occurring within 48 hours after administration of a single dose of a drug were considered to be due to its action. The occasional death which occurred several days after a single dose of a drug was considered to be due to extraneous factors. The LD 50 was approximated graphically.

The analgetic action of these drugs was evaluated by a modification of the method developed by D'Amour and Smith (6). This method was chosen because it seems convenient for small animals and thus facilitates the use of adequate numbers to allow for individual variation. This procedure measures the duration of a constant-intensity heat-stimulus required to produce a response when applied to a rat's tail. The heat from a 50 candle-power incandescent light is concentrated by an appropriate reflector and focused by a lens on the terminal portion of the rat's tail. The response taken as the end-point is a characteristic twitch easily recognized after short experience.

[1] Supported in part by a grant from Frederick Stearns and Company for research in Pharmacology.

3. Eagle, H., Magnuson, H. J., and Fleischman, R.: J. Clin. Invest., 25: 451, 1946.
4. Eagle, H.: J. Ven. Dis. Inf., 27: 114, 1946.
5. Eagle, H., Magnuson, H. J., and Fleischman, R.: Am. J. Syph., 30: 420, 1946.
6. Braun, H. A., Lusky, L. M., and Calvery, H. O.: Supplement to This Journal, 87: 119, 1946.
7. Maren, T.: Bull. J. H. H., 77: 338, 1945.
8. Freedman, L. D.: Ind. & Eng. Chem., Anal. Ed., in press.
9. Leutscher, J. A., Eagle, H., and Longcope, W. T.: J. Clin. Invest., 25: 534, 1946.
10. Wexler, J., Eagle, H., Tatum, H. J., Magnuson, H. J., and Watson, E. B.: J. Clin. Invest., 25: 467, 1946.
11. Hogan, R. B., and Eagle, H.: This Journal, 80: 93, 1944.
12. Doak, G. O., and Steinman, H. G.: J.A.C.S., 68: 1987, 1946.

once daily, six days per week by the same technique used for other studies. When acetylsalicylic acid was given in a dose of 0.9 gm./kgm./day, fourteen of the fifteen rats used died following the second dose. The fifteenth animal was not studied further. When the dosage was reduced to 0.7 gm./kgm./day, five of the fifteen rats used died following the third dose (one additional animal died at this time as a result of accidental administration of the drug suspension into the lung), three animals died following the fourth dose, three following the

TABLE 1

Comparative toxicity and analgetic potency of the salicylates and some other analgetic compounds

LD 50 is the amount required to kill 50 per cent of a large group of animals (calculated from groups given various doses). AD 50 is the amount required to produce significant analgesia in 50 per cent of a group of animals (calculated from groups given various doses). Fifteen or more animals were used at each dose except where otherwise indicated.

COMPOUND	ANIMAL	LD 50	AD 50
		gms./kgm.	*gms./kgm.*
Sodium salicylate	Mouse	0.9	—
	Rat	1.6	0.13
	Rabbit (6/dose)	1.7	—
Acetylsalicylic acid	Mouse	1.1	—
	Rat	1.5	0.35
	Rabbit (12/dose)	1.8	—
Salicylamide	Mouse	1.4	—
	Rat	1.4	0.06
	Rabbit (6/dose)	3.0*	—
O-Acetyl salicylamide	Rat (10/dose)	1.8	0.13†
N-Acetyl salicylamide	Rat (10/dose)	2.0	0.06†
Bromosalicylamide	Rat (10/dose)	4.0*	0.06†
Diiodosalicylamide	Rat (10/dose)	1.4*	0.06†
Antipyrine	Rat (10/dose)	1.8	0.22
Aminopyrine	Rat	1.7	0.75‡
Acetanilid	Rat	1.7	0.18
Acetophenetidin	Rat	2.6	0.56

* Less than 50 per cent killed by this dose. Larger doses not used (see text).

† More than 50 per cent showed significant analgesia at this dose. Smaller doses not used because of scarcity of material.

‡ Less than 50 per cent showed significant analgesia at this dose. Larger doses not used because of toxicity.

fifth dose, one following the twelfth dose, and the remaining two survived a total of 29 doses at which time the experiment was discontinued. Further reduction of the dosage to 0.5 gm./kgm./day resulted in more prolonged survival; four of the fifteen rats died after the second dose, one after the third dose (one more died at this time from faulty administration), one after the sixth dose, and the remaining eight survived a total of 19 doses at which time the experiment was discontinued.

Our modification of the method consists in arranging the apparatus so that the animal is exposed to a warmth stimulus before the pain stimulus is applied. This is accomplished by including a suitable resistance in the circuit and arranging switches so that the light burns at reduced intensity until the resistance is short-circuited and the high intensity stimulus applied. The timer operates only while the pain stimulus is being applied. The resistance is so adjusted that the animal will tolerate the warmth indefinitely. We believe that this modification increases the reliability of the results by permitting the rat to become adapted to a sensation of warmth and thus eliminating premature response to the heat stimulus.

A normal, untreated rat will respond to a stimulus applied as described in some 3 to 5 seconds and the response time will vary somewhat in consecutive trials. The average response time of our rats is in essential agreement with that reported by D'Amour and Smith (6), but they found the variability so low as to be negligible. We have not found response times to be reproducible from rat to rat nor in the same rat from time to time and do not feel justified in neglecting this factor. Analysis of data obtained on a large number of normal rats has shown that, with our procedure, consecutive observations on the same rat made at about 30 minute intervals will usually fall within plus or minus 1.7 seconds of the average of those observations. Therefore we consider that an increase in response time of at least 2.0 seconds is required to indicate significant analgesia in a given animal. This criterion of analgesia is admittedly a severe one, but it seems desirable since by its use we err on the conservative side if at all.

To make allowance for these factors we have adopted the following procedure for testing analgetics. Three control observations are made (at about 30 minute intervals) on each rat on the day of the experiment; the animal is then given the drug being studied; and additional observations are made 15, 45, 75 and 105 minutes after the drug was given. The three control response times are averaged and this figure compared with each of the four response times observed after the drug was given. If any one of the four exceeds the control average by 2.0 seconds or more that rat is considered to have shown significant analgesia. The incidence of analgesia in the group of rats is then tabulated as if the response were all-or-none. From several groups handled similarly the dose of the drug required to produce analgesia in 50 per cent of the animals (AD 50) is then approximated graphically.

Results. The results of the toxicity experiments are given in table 1. All compounds were not tested on mice and rabbits for reasons of economy of both drugs and animals. It will be seen that sodium salicylate, acetylsalicylic acid and salicylamide show surprisingly little variation in toxicity for the three species studied, except for the seeming lack of toxicity of salicylamide for rabbits. This lack of toxicity may well be an artifact due to failure of absorption or some other cause. It was not feasible to give higher doses of salicylamide to the rabbits because the bulk of the dose in a usable suspension approached the capacity of the stomach.

Except for the halogenated salicylamides, all the compounds studied appear to be similar in their toxicity for rats. The halogen derivatives were not studied further because of inadequate supply of the compounds.

The intoxication syndrome following administration of salicylamide or its derivatives seemed to differ in one important respect from that following the other drugs. All of the established analgetics produced convulsions prior to death while the amides seemed to have a depressant (or possibly paralytic) action.

The chronic toxicity of acetylsalicylic acid and of salicylamide was studied by the administration of moderately large doses (35–65% of the LD 50) to rats

SUMMARY AND CONCLUSIONS

1. When administered to rats by stomach tube the LD 50 (gms./kgm.) for sodium salicylate is 1.6, acetylsalicylic acid 1.5, antipyrine 1.8, aminopyrine 1.7, acetanilid 1.7, acetophenetidin 2.6, salicylamide 1.4, O-acetyl salicylamide 1.8, N-acetyl salicylamide 2.0, bromosalicylamide greater than 4.0 and diiodo-salicylamide greater than 1.4.

2. When administered to mice by stomach tube the LD 50 (gms./kgm.) for sodium salicylate is 0.9, acetylsalicylic acid 1.1 and salicylamide 1.4.

3. When administered to rabbits by stomach tube the LD 50 (gms./kgm.) for sodium salicylate is about 1.7, acetylsalicylic acid about 1.8 and salicylamide above 3.0.

4. Salicylamide is considerably less toxic than acetylsalicylic acid when repeated doses are given at daily intervals.

5. Using a modification of the D'Amour and Smith procedure the analgetic potencies of salicylamide and its derivatives appear to equal or exceed those of the established analgetic-antipyretic drugs.

We wish to acknowledge our indebtedness to Dr. Thomas M. Scotti of the Department of Pathology for his cooperation. We are also indebted to Mrs. Shirley Busser for technical assistance.

REFERENCES

(1) Houben, J.: Fortschritte der Heilstoffchemie, Abt. II, vol. 2, page 562.
(2) Gérard, E., and Leroy, J.: Jour. de Pharmacie et de Chimie, (7) 5: 329, 1912.
(3) Gonnermann: Arch. ges. Physiol., 89: 493, 1902.
(4) St. Bondzynski: Arch. exp. Path. Pharmakol., 38: 98, 1897.
(5) Baas, H. K. L.: Zeit. physiol. Chem., 14: 431, 1890.
(6) D'Amour, F. E., and Smith, D. L.: This Journal, 72: 74, 1941.
(7) Nebelthau, E.: Arch. exp. Path. Pharmakol., 36: 456, 1895.

Administration of salicylamide at a dosage level of 0.9 gm./kgm./day resulted in the death of two rats after the ninth dose, one after the twelfth, and one after the nineteenth dose. The remainder of the fifteen rats used survived a total of 36 doses except for five animals which died as a result of faulty administration after the third, fourth, fifth, tenth and twenty-fourth doses. When this compound was given at a dosage level of 0.7 gm./kgm./day, one rat died after the nineteenth dose and six survived a total of 36 doses. Six additional rats in this group died from faulty administration after the second, third, eleventh, fourteenth, seventeenth and twenty-ninth doses.

Microscopic examination of the tissues of animals which died following repeated administration of either acetylsalicylic acid or salicylamide showed hyperemia throughout the tissues and edema and alveolar hemorrhages of the lungs. One animal which had received salicylamide showed multiple foci of necrosis with cellular infiltration in the liver and spleen. The significance of these findings is not clear at this time.

The analgetic potency of the various compounds is also given in table 1. It may be seen that the AD 50s for salicylamide and for its diiodo derivative are approximately half the corresponding figures for the most potent of the established analgetics. The other amides do not seem to be significantly different from the established compounds.

Discussion. Salicylamide and those of its derivatives which have been adequately studied do not seem to differ appreciably from the established analgetic-antipyretics in acute toxicity. In contrast it appears that salicylamide is considerably less toxic than acetylsalicylic acid on repeated administration. We are unable to explain this, but it is probably due to some difference in action not observable in simple toxicity experiments.

Similarly we cannot explain the difference between the toxic symptoms produced by the amide derivatives and by the other compounds. There is no consistent difference in chemical structure to which the difference in action can be attributed. Nebelthau (7) reported that aromatic acid amides have an "alcohol-like narcotic action" which is in agreement with our findings. However he did not compare amides with other types of compounds. Whatever the explanation, the fact remains that the amides produced a depressant type of action while the other compounds all produced convulsions.

A wide variety of ingenious methods for testing analgetics has been proposed using a variety of animal species. We do not contend that the method used in this study is necessarily more reliable than many others. Whatever the defects of our method may be, by its use we have not yet failed to detect analgetic action in a drug which has proven clinically useful for the relief of pain.

Since we are unable to define the limitations of our method we do not feel justified in attempting to rank the established drugs according to analgetic potency. However the analgetic potencies of salicylamide and its derivatives seem to indicate that this type of compound should not be neglected in any search for useful analgetics.

and Witherup (6), these animals excrete smaller quantities of glucuronic acid and carbohydrate than on mixed or protein diets. Our results for excreted glucuronic acid are expressed in mgm. per Kg., body weight daily (24 hours) whereas Deichmann's are expressed in mgm. in 24 hours regardless of body weight. However, the majority of our control values agreed remarkably well, when his values are divided by body weights of 2 to 3 Kg.

The pertinent details are summarized in Table 1 and the essential results may be summarized as follows: The 7 rabbits that received propylene glycol showed

TABLE 1

Urinary excretion of glucuronic acid after diethylene glycol monoethyl ether and some other glycols in rabbits

RABBIT	GLYCOL† USED	DOSE CC. PER KG.	METHOD OF ADMINISTRATION	GLUCURONIC ACID EXCRETION (MG. PER KG. DAILY)					TOTAL PER CENT INCREASE IN GLUCURONIC ACID EXCRETION OBSERVED
				Average before glycols	After glycols				
					First day		Second day		
					mg. per kg.	per cent increase	mg. per kg.	per cent increase	
183	PG	5.0	Hypodermic	13(3)*	93	615	34	162	388
184	PG	5.0	Hypodermic	12(3)	154	1183	56	367	775
185	PG	10.0	Hypodermic	48(3)			416	767	333
199	PG	10.0	Hypodermic	22(3)	45	103	70	218	161
200	PG	10.0	Hypodermic	22(2)	71	223	7	0	80
240	PG	2.3	Gastric	9(2)	11	22	0	0	22
241	PG	2.3	Gastric	16(3)	32	100	14	0	100
185	DEGM	3.0	Hypodermic	46(2)	82	78	Died		78
186	DEGM	3.0	Hypodermic	14(2)	18	22	110	686	358
188	DEGM	5.0	Hypodermic	33(2)	75	127	Died		127
197	DEGM	4.0	Gastric	8(3)	78	875	Died		875
198	DEGM	4.0	Gastric	17(3)	84	394	35	106	250
206	EG	4.0	Gastric	29(3)	52	79	19	0	22
207	EG	4.0	Gastric	17(3)	13	0	10	0	0
208	DEG	4.0	Gastric	15(3)	15	0			0
209	DEG	4.0	Gastric	23(1)	32	39			39
242	DEG	2.0	Gastric	19(1)	19	0			0
243	Glycerol	4.0	Gastric	9(1)	11	10	11	10	11
200	Glycerol	4.0	Gastric	30(2)	16	0	12	0	0

* Digits in parentheses indicate number of days during which determinations were made.

† Symbols in this column are for the following agents: PG, propylene glycol; DEGM, diethylene glycol monoethyl ether; EG, ethylene glycol; DEG, diethylene glycol.

increases in the excretion of glucuronic acid ranging from 22 to 1183 per cent over the average daily excretion before the drug, on the first day, and from 0 to 367 per cent on the second day after the glycol; total for both days, 22 to 775 per cent. With diethylene glycol monoethyl ether the results in 5 rabbits were similar during the first day, or an excretion of 22 to 875 per cent, but the majority of animals died before the end of the second day, the total excretion under the conditions being 78 to 875 per cent. The considerable range of excretions after both agents was uninfluenced by their dosage; in fact, there were considerable

GLUCURONIC ACID EXCRETION AFTER DIETHYLENE GLYCOL MONOETHYL ETHER (CARBITOL) AND SOME OTHER GLYCOLS*

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Received for publication November 2, 1946

In determining the fate *in vivo* of diethylene glycol monoethyl ether (carbitol), which is widely used in cosmetic and industrial products and limitedly in dermatological formulas, it was found that only a relatively small percentage was excreted in the urine regardless of the route of administration (1). Most of it appeared to be readily disposed of, i.e., probably destroyed, but the possibility remained that the undestroyed portion was excreted in conjugated form as a glucuronate, as is the case with propylene glycol. For instance, Neubauer (2) found that propylene glycol given to rabbits increased glucuronic acid in urine, while glycerol did not. Miura (3) reported that ethylene glycol and glycerol in rabbits did not change the daily excretion of glucuronates, while a small percentage of propylene glycol was excreted conjugated with glucuronic acid. Miura isolated the barium salt of this glucuronate. Since the results of these authors indicated differences in the metabolism of propylene glycol and ethylene glycol and we have reported other evidences of a similarity in systemic behavior of propylene glycol and diethylene glycol monoethyl ether (1), it appeared desirable to see if this similarity existed in their metabolic actions for a further understanding of the fate of the ether in the body. Therefore, we have compared the effects of glycerol, ethylene glycol, diethylene glycol, propylene glycol and diethylene glycol monoethyl ether on the daily excretion of glucuronic acid in rabbits, using the results with the first 4 compounds as controls on those with the ether.

PROCEDURE. The rabbits were kept on a diet of rolled barley for 4 or 5 days before starting the collection of the control urine samples, which were obtained by catheterization during 24 hours. Control samples were collected for 1 or 4 days, and then the compound was administered and the urine again collected for 24 hours during 2 days. The following were given gastrically: ethylene glycol and glycerol each in single doses of 2 to 4 cc. per Kg. with 100 cc. of water, and diethylene glycol in doses of 2 to 4 cc. per Kg. Propylene glycol was given in single doses of 10 cc. and 5 cc. per Kg. hypodermically and 2.3 cc. per Kg. gastrically. Diethylene glycol monoethyl ether was administered hypodermically in doses of 3 and 5 cc. per Kg. and gastrically in doses of 4 cc. per Kg. The doses of diethylene glycol, ethylene glycol and diethylene glycol monoethyl ether used by us were within the fatal range, and some animals died on the second day after administration.

The method used for the quantitative estimation of glucuronic acid was that described by Deichmann (4), a modification of Tollen's (5) original method with naphthoresorcinol. We are indebted to Dr. Deichmann for courteous suggestions regarding use of his method and for supplies of glucuronic acid monobenzoate and the borneol ester of glucuronic acid as standards. The rabbits were kept on a diet of rolled oats, since, according to Deichmann

* Preliminary report by Fellows, Jean K., and Luduena, F. P.: Fed. Proc., 1946, 5: 178.

2. Why carbitol, a member of the ethylene series of glycols, should differ in this respect from the ethylene and diethylene glycols, which are chemically somewhat different, is not clear, but carbitol and propylene glycol are fairly readily disposed of in the body, while the other glycols are not, at least in critical doses or concentrations. Various considerations are discussed.

REFERENCES

1. Luduena, F. P., Lawrence, W. S., Fellows, Jean K., Clark, W. H., and Hanzlik, P. J.: (In press).
2. Neubauer, O.: Arch. exptl. Pathol. Pharmakol., 46, 133, 1901.
3. Miura, S.: Biochem. Zeitschr., 36: 25, 1911.
4. Deichmann, W.: J. Lab. and Clin. Med., 28: 770, 1943.
5. Tollens: *Handb. der Kohlenhydrate.* Ed. 3, Leipzig, 1914.
6. Deichmann, W. B., and Witherup, S.: Arch. of Biochem., 7: 401, 1945.
7. Meininger, W. M., Fellows, Jean, and Luduena, F. P.: (To be published).
8. Hanzlik, P. J., Lehman, A. J., Van Winkle, Jr., W., and Kennedy, N. K.: J. Pharm. Exp. Therap., 67: 114, 1939.
9. Deichmann, W. B., Kitzmiller, K. V., and Witherup, S.: Arch. of Biochem., 7: 409, 1945.

variations on the same dosage. With ethylene glycol there was only a small increase (79 per cent) in one animal during the first day, and no increase in the other. Diethylene glycol and glycerol produced no increases in 5 animals, 2 small amounts falling within range of experimental error.

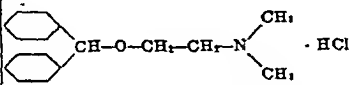
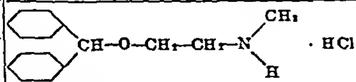
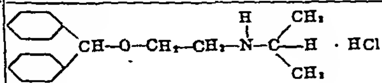
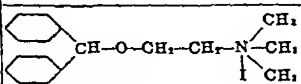
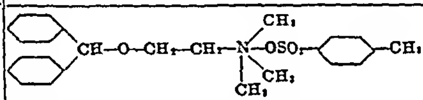
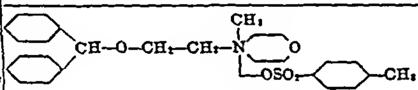
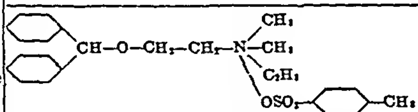
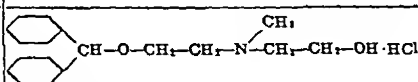
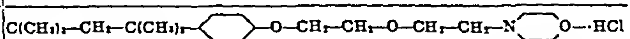
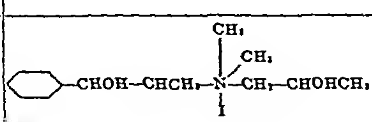
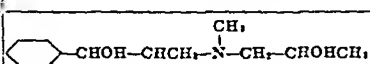
Discussion. The considerable, though variable, increases in glucuronic acid excretion after the large and mostly toxic doses used of diethylene glycol monoethyl ether suggest that this agent appears in the urine conjugated with glucuronic acid, as is apparently the case with propylene glycol. If this is true, and assuming that one molecule of the ether combines with one molecule of glucuronic acid, the amount excreted in this way would represent only a very small percentage (0.8 to 2.3 per cent) of the total amounts administered. Presumably, the greater part of the diethylene glycol monoethyl ether is oxidized in the body, in agreement with the low quantitative excretion in rabbits previously demonstrated with different methods of administration (1). No excretion of glucuronic acid above control levels was demonstrable in human individuals after epidermal applications of ointments and lotions containing 20 to 50 per cent of diethylene glycol monoethyl ether which agreed with the negative excretions of the ether itself in these urines (7).

Our results with propylene glycol, ethylene glycol and glycerol confirm those of Neubauer and Miura. They indicate that a derivative of the ethylene glycol series can undergo the same fate, in part at least, as a compound of the propylene series. It is assumed, with others, that increases in urinary glucuronic acid signify conjugation resulting from the administration of foreign agents, but it must be granted that the 2 positively acting glycols may produce glucuronate by some other metabolic action. Whatever be the correct mechanism, it is interesting that of all the glycols thus far examined, the 2 which are rather readily disposed of in the body, without serious systemic injuries at certain levels, are characterized by increases in glucuronate excretion (detoxication by conjugation?) whereas the other glycols are not. It is also interesting that the glycols of the ethylene series are not glycogenic, but propylene glycol and glycerol are (8). Whether the 2 metabolic changes, i.e., glucuronic conjugation and glycogenesis, are related is not known. Any possible visceral injuries caused by the diethylene glycol monoethyl ether in our animals, especially those that lived 2 days, did not interfere with glucuronate excretion, which would be in general agreement with the results of Deichmann, Kitzmiller and Witherup (9) who found no interference by hepatic injuries caused by phosphorus, chloroform and carbon tetrachloride.

CONCLUSIONS

1. Diethylene glycol monoethyl ether (carbitol) and propylene glycol administered in large doses gastrically or hypodermically to rabbits produce similar though variable and sometimes considerable increases in the daily urinary excretion of glucuronic acid, while ethylene glycol, diethylene glycol and glycerol administered similarly do not, results with the latter groups and propylene confirming those of others.

TABLE 1

CODE NUMBER	CHEMICAL NAME	FORMULA
S-51	B-dimethylaminoethyl benzhydryl ether (Benadryl)	
S-59	B-monomethylaminoethyl benzhydryl ether	
S-52	B-isopropylaminoethyl benzhydryl ether	
S-92	B-benzhydryloxyethyl trimethylammonium iodide	
S-154	B-benzhydryloxyethyl trimethylammonium p-toluenesulfonate	
S-157	4-B-benzhydryloxyethyl-4-methylmorpholinium p-toluenesulfonate	
S-158	B-benzhydryloxyethyldimethylethyl ammonium p-toluenesulfonate	
S-161	B-benzhydryloxyethyl-B-hydroxyethyl-methylamine	
S-150	N-(p-tertiary-octylphenoxyethoxyethyl) morpholine	
S-164	N-B-hydroxypropylephedrine methiodide	
S-165	N-B-hydroxypropylephedrine (free base)	

measured as cc. per minute and any pair of lungs which failed to attain a relatively constant control perfusion rate was discarded.

A COMPARISON OF THE BRONCHODILATOR ACTIVITY OF BENADRYL WITH SOME OF ITS DERIVATIVES AND CERTAIN OTHER SUBSTANCES[1]

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Received for publication November 12, 1946

There is available a considerable amount of data to support the current theory that anaphylactic and allergic manifestations are due to histamine. Consequently, numerous attempts have been made to find a substance that might be efficient in neutralizing the effects of histamine in the body, and thereby, afford a useful therapeutic agent. The literature in this field has been reviewed previously by Lehmann and Young (1) and by Loew et al. (2).

Several papers have appeared recently dealing with the ability of certain benzhydryl ethers to antagonize histamine and anaphylactic phenomena. In an earlier report from this laboratory it was shown that B-dimethylaminoethyl benzhydryl ether (Benadryl) and B-monomethylaminoethyl benzhydryl ether were effective in antagonizing histamine and anaphylactic bronchoconstriction in excised guinea pig lungs (3). Loew and his co-workers reported that of a group of benzhydryl alkamine ethers investigated, Benadryl was the most efficacious in preventing fatal experimental asthma in guinea pigs by the administration of atomized histamine solution (2). These workers further demonstrated that Benadryl was equally effective in alleviating anaphylactic shock in guinea pigs (4).

The purpose of the present investigation was to compare the bronchodilator activity of Benadryl with certain of its analogs in perfused isolated guinea pig lungs and on *in situ* bronchial muscles in dogs. Three other compounds, chemically unrelated to Benadryl, were included in this study because of their possible spasmolytic property. All of the agents considered in this paper are members of a general series of compounds synthesized in an effort to find a good spasmolytic drug. The chemical formulas of these "S" compounds are shown in table 1. For convenience, with the exception of Benadryl, they will be referred to in the text by the code numbers appearing in the table. An abstract of this work has appeared previously (5).

ISOLATED LUNGS. Excised lungs from guinea pigs weighing 200 to 300 grams were perfused according to Tainter's modification (6) of the Sollmann-von Oettingen method (7). The perfusion fluid was delivered from a pressure head of 20 cm. and circulated through a constant temperature water bath before reaching the lungs. The lung surface was scarified to give a perfusion rate of approximately 50 cc. per minute. The outflow of fluid was

[1] This investigation has been supported by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association and from Parke, Davis and Company, Detroit, Michigan.

Compound S-82 failed to completely inhibit constriction in only 2 lungs while in the remainder of the experiments a definite dilatation occurred which varied between 2.0 and 19.0 per cent increase over the control rate. Compounds S-92 and S-158 appeared to be about equal in anti-histamine effect but neither was as efficient as Benadryl. The effectiveness of S-154 and S-157 was pronounced but complete suppression of histamine constriction was not demonstrated consistently. Partial antagonism to the bronchoconstriction was exhibited by S-161 but this compound was the least potent of the Benadryl derivatives.

The relative inefficiency of S-150 may be attributable to the fact that precipitation occurred each time this compound became mixed with the perfusion fluid. However, it should be pointed out that this member of the series is not a chemical derivative of Benadryl and, therefore, may not possess the anti-histamine effect to any marked degree. Compounds S-164 and S-166, two other members of the group chemically unrelated to Benadryl, failed to show any significant inhibition to histamine.

Several experiments were carried out in which the three spasmolytic compounds most active against histamine were used to antagonize barium and pilocarpine constriction. The procedure was similar to that described above for histamine. Twenty-five mgm. (1 cc. of 2.5 per cent) of barium chloride and 1 mgm. (1 cc. of 1:1000) of pilocarpine nitrate were the standard doses employed. The amount of constriction induced by these drugs was approximately the same as with histamine. Each of the three bronchodilators, when injected with barium or pilocarpine, completely suppressed spasm of the lungs and, in addition, gave a dilatation over and above the control measurements. Against barium, average increases in perfusion rates were as follows: Benadryl, 7.7 per cent; S-59, 7.9 per cent; and S-82, 23.3 per cent. When pilocarpine was used the averages were: Benadryl, 7.8 per cent; S-59, 16.6 per cent; and S-82, 13.1 per cent.

Hence, it appears from these experiments that Benadryl and some of its derivatives not only possess an anti-histamine effect but, moreover, act as spasmolytic agents against other constrictors.

In situ lungs. Using the results obtained on excised bronchi as an indication of the relative bronchodilator activity, Benadryl, S-59, S-82, S-92, S-154, S-157 and S-158 were selected for further study on dog lungs *in situ*. Fifty dogs, weighing from 7 to 19 kgm., were used in this phase of the investigation. Fifteen of these animals were anesthetized with ether and then the brain and medulla were pithed. The remaining animals were anesthetized with pentobarbital sodium, 30 mgm. per kgm. and dihydro-beta-erythroidine[2] was given in 10 mgm. per kgm. doses as needed to paralyze spontaneous respiratory movements. Bronchial tonus was recorded by Jackson's method (8) using negative artificial respiration. A mercury manometer was connected to the left carotid artery for recording blood pressure. Both vagi were cut in all of the animals. Histamine (1:1000) was the only agent used to produce bronchoconstriction. The bronchodilators were used in the same concentrations as previously stated All injections were by the intravenous route into the femoral vein.

[2] Supplied through the courtesy of Merck and Company, Rahway, N. J.

In order to test the bronchodilator activity of the spasmolytic agents, it was necessary to produce an initial constriction in the lungs. This was done by injecting into a rubber tubing leading to the trachea 0.1 mgm. (1 cc. of 1:10,000) of histamine. The amount of spasm thus produced was calculated on the basis of percentage decrease from the initial outflow and was taken as the control constriction for any given experiment. Under these conditions, the constriction varied from 30 to 85 per cent. Sufficient time then was allowed for recovery and in all but a few cases, the outflow returned practically to the control rate of perfusion. Thereupon, the standard dose of histamine plus 10 mgm. of the compound to be tested were injected simultaneously. All of these drugs except one were used as 1 per cent solutions. Owing to limited solubility, S-92 was employed as a 0.25 per cent solution. Following this injection, the outflow was measured until the altered rate became maximal and a return toward the control rate was evident. In each experiment, subsequent doses

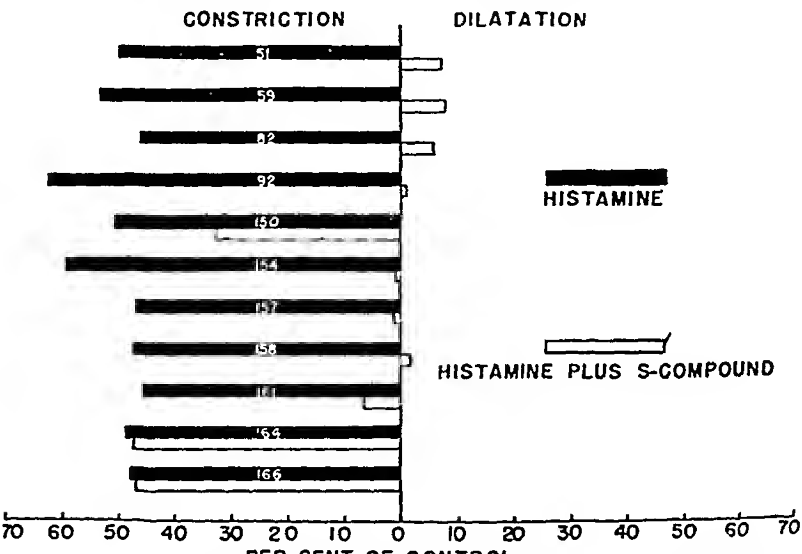

FIG. 1. AVERAGE RESULTS OF EXPERIMENTS ON ISOLATED PERFUSED GUINEA PIG LUNGS

Black bars represent construction of 0.1 mgm. of histamine alone; white bars represent combined effects of 0.1 mgm. of histamine plus 10 mgm. of "S" compounds. White numbers on black bars are designations of "S" compounds. S-51 is Benadryl.

of the constrictor agent were used to test for continued responsiveness of the bronchial musculature. From 4 to 12 pairs of lungs were used for each compound and 90 lungs were used in the total study.

The results obtained are compiled as averages and are presented in chart form in figure 1. It will be seen from this chart that Benadryl, S-59, S-82, S-92 and S-158 when injected with the standard dose of histamine, not only prevented bronchial constriction but effected a superimposed dilatation. Of these five compounds, only Benadryl and S-59 produced an increase over the control outflow in each experiment performed. This increase varied from 1.7 to 14.7 per cent with Benadryl and ranged from 1.9 to 17.0 per cent in the case of S-59.

The other approach to this problem involved experiments in which an attempt was made to estimate the relative duration of antihistamine effect when the S-compounds were given *before* histamine. In this procedure 2 to 5 mgm. per kgm. of the bronchodilator were injected first and this was followed 5 minutes later by a standard dose of 0.1 mgm. per kgm. of histamine. If no constriction resulted from the latter, subsequent similar doses of histamine were injected at 15 to 30 minute intervals until a definite degree of constriction occurred.

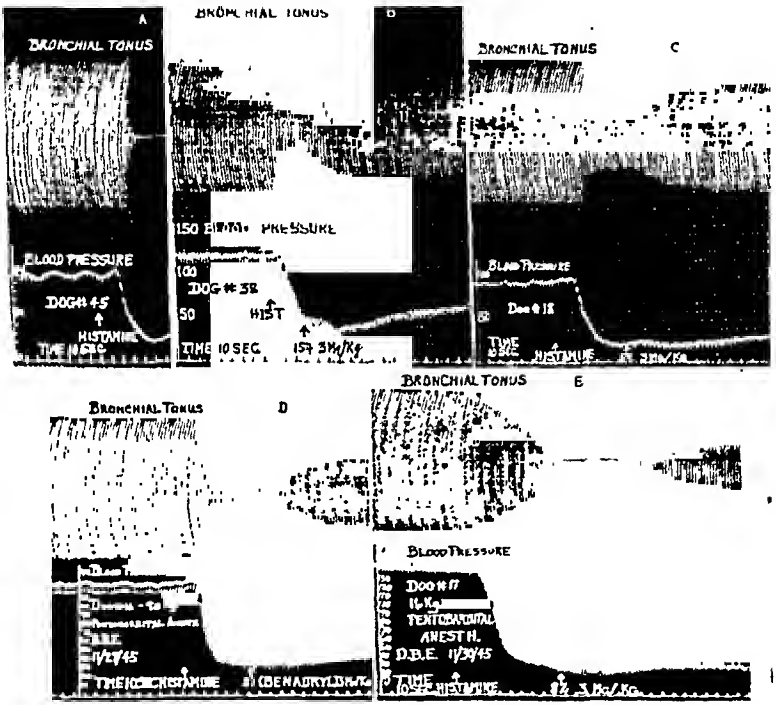

FIG. 2. COMPARISON OF BRONCHODILATOR EFFICIENCY OF BENADRYL DERIVATIVES
Bronchial constriction is indicated by a decrease in amplitude of respiratory excursion. Pentobarbital anesthesia; paralysis of spontaneous respiratory movements with dihydro-beta-erythroidine; histamine, 0.1 mgm. per kgm.

Control experiments indicated that no significant amount of tolerance developed to these repeated doses of histamine.

All of the compounds used prevented bronchoconstriction following the first injection of histamine. However, the duration of this protective effect varied over a wide range of time depending upon the compound and dose employed. The data collected on this phase of the work are shown in table 2. Although in most cases only one experiment was performed at a given dosage level for each compound, the composite results obtained were consistent within each

Two methods of approach were used in studying the effects of these compounds on bronchospasm. In one procedure, an initial constriction was induced by histamine in doses varying from 0.05 to 0.2 mgm. per kgm. This range of dosage produced severe bronchial spasm which led to death of the animal unless the constriction was opposed by one of the bronchodilators. When the immediate maximum effects appeared, 2.0 to 5.0 mgm. per kgm. of the S-compound were injected and the degree of bronchial dilatation was noted. All of the dilators gave some degree of relief from the bronchoconstriction but there was a wide variation in this effect. In the doses employed, all of the S-compounds produced a fall in blood pressure and, consequently, when this effect was superimposed upon the histamine vasodepression, an acute circulatory embarrassment followed. In many instances, it is possible that this depression of the circulation prevented the dilator from exerting the maximum beneficial effect on the bronchial muscles. In a few experiments the severe fall in blood pressure lead to death of the animal in spite of the fact that an appreciable degree of bronchodilatation occurred. This condition prevailed especially following the use of S-82.

Although there are many variable factors in this method, and consequently, no exact quantitative measurement is permissible, an attempt was made to compare the bronchodilator efficiency of Benadryl with that of several of its derivatives using the constant dosage of 3 mgm. per kgm. Typical records demonstrating this comparison are shown in figure 2. Only those compounds showing the most potent and the least potent anti-histamine effects are included here for the purpose of illustration. In view of the initial bronchial tonus and the degree of constriction produced by histamine, the best dilators for instant alleviation of bronchoconstriction were S-154, S-59 and Benadryl. It is difficult to make a clear cut distinction between the immediate effects of these three compounds. However, S-154 seemed to produce a greater degree of relaxation in a shorter period of time than did either S-59 or Benadryl. Furthermore, S-59 usually showed a more marked bronchodilatation within the first 2 minutes than was the case with Benadryl. This general relationship is apparent when records 2B, 2C and 2D are compared. Nevertheless, Benadryl was quite consistent in affording good relief from bronchial spasm and after full development of the effect of this drug, the duration of action was longer than that of any of the other compounds.

The least effective compound in producing prompt relief from the histamine constriction was S-82. Although some dilatation followed the use of this derivative, the onset of action was delayed beyond the point of benefit in many of the animals. Several of the animals died in spite of treatment with this agent. A typical response is shown in figure 2E.

Compounds not represented in figure 2 had effects varying between Benadryl and S-82 in the following order of decreasing activity: S-92, S-157 and S-158. Each of these three agents antagonized the bronchial spasm sufficiently to sustain the animals but rarely did the bronchial tonus completely return to the control height.

SUMMARY

A comparison of the bronchial antispasmodic action of Benadryl, some of its derivatives and certain other substances has been made in isolated lungs and on bronchial muscle *in situ*.

In the isolated perfused guinea pig lungs, Benadryl and its monomethyl (S-59) and isopropyl (S-82) derivatives were the most effective compounds in preventing histamine constriction. Furthermore, these three compounds caused an additional dilatation of the lungs even in the presence of a constrictor dose of histamine. The other compounds investigated varied in effect from practically no antagonism to complete prevention of the histamine constriction.

On dogs' bronchi *in situ*, S-154 and S-59 appeared to relieve previously induced histamine bronchospasm faster than Benadryl. However, Benadryl was more consistent and reliable in producing bronchodilatation. S-82 was the least effective compound in relieving acute bronchial spasm.

The duration of action of the most effective S-compounds, following their injection prior to histamine, may be compared as follows: Benadryl > S-59 > S-82 > S-154.

The other agents investigated (S-92, S-157 and S-158) were either weak in immediate antagonism to histamine or had a very short duration of action.

REFERENCES

1. LEHMANN, G., AND YOUNG, JAMES W.: THIS JOURNAL, 83: 86, 1945.
2. LOEW, EARL R., KAISER, MARGARET E., AND MOORE, V.: THIS JOURNAL, 83: 120, 1945.
3. ELLIS, FRED W.: Federation Proc., 4: 117, 1945.
4. LOEW, EARL R., AND KAISER, MARGARET E.: Proc. Soc. Exper. Biol. Med., 58: 235, 1945.
5. ELLIS, FRED W., AND NEWSOME, JAMES F.: Federation Proc., 5: 176, 1946.
6. TAINTER, M. L., PEDDEN, J. R., AND JAMES, M.: THIS JOURNAL, 51: 371, 1934.
7. SOLLMANN, T., AND VON OETTINGEN, W. F.: Proc. Soc. Exper. Biol. Med., 25: 692, 1928.
8. JACKSON, D. E.: Experimental Pharmacology and Materia Medica. 2nd Edition, 1939.

series. Hence, an analysis of the total number of experiments, rather than any single one, conducted with each S-compound gives a fair indication of the relative length of time during which protection against histamine bronchospasm is effective.

It will be seen from table 2 that the effect of Benadryl far exceeded that of any other dilator in this group. This is true regardless of the dosage used. On the other hand, S-154 which gave the most rapid recovery from induced spasm (fig. 2B), furnished protection against histamine for a much shorter period of time.

TABLE 2

Duration of action of benadryl derivatives

COMPOUND	DOSE	NUMBER OF EXPERIMENTS	DURATION
	mgm per kgm		*minutes*
Benadryl	2	1	70
	3	1	90
	5	3	125*
S-59	2	1	20
	3	2	30*
	5	1	80
S-82	2	1	20
	3	2	30*
	5	1	70
S-154	3	1	35
	5	1	60
S-92	2	1	15
	3	1	20
	5	1	30
S-157	5	2	20*
S-158	5	2	15*

* Average.

Another comparison which might be pointed out is the close similarity in duration of action between S-59 and S-82. S-59 seemed to be more effective in only one experiment. In view of the relative ineffectiveness of S-82 in relieving previously induced bronchoconstriction (figure 2E), it is interesting to note that this compound showed remarkable bronchodilator activity when injected prior to histamine.

The duration of action of S-92, S-157 and S-158 varied from 15 to 30 minutes and, in general, these compounds were judged to be inferior to the agents discussed above.

cholesterol before the pentothal was injected Pentothal sodium was injected intravenously, 0.5 cc /kg of a 2 5 per cent solution The cholesterol was injected intraperitoneally, 2 4 cc /kg of a 12 5 per cent suspension in 6 per cent acacia solution

The data are given in table 2. They show, when subjected to statistical[4] analysis, that in the dog, as in the rabbit, injections of cholesterol significantly lengthen the anesthesia period with pentothal. From the standard errors of the two series of data the variation observed would occur only once in a thousand trials by chance.

TABLE 1

The effect in rabbits of the intravenous injection of sodium pentothal after the intravenous administration of cholesterol

SUBSTANCE ADMINISTERED	NO OF RABBITS	AV WT	ANESTHESIA DURATION
		kg	*min*
	25	2 14	5.68 ±0.45
2 5 cc /kg 25% Carbowax	15	2 01	5 75 ±0 71
2 5 cc /kg of a 2% suspension of cholesterol in 25% Carbowax solution	13	2.00	16 72 ±2.23

TABLE 2

The effect in dogs of the intravenous administration of sodium pentothal before and after the intraperitoneal administration of cholesterol

DOG	WT	SEX	CONTROL ANESTHESIA	ANESTHESIA (MIN) AFTER 2 4 CC /KG OF A 12.5% SUSPENSION OF CHOLESTEROL IN 6% ACACIA	PER CENT CHANGE IN DURATION OF ANESTHESIA OWING TO CHOLESTEROL
	kg		*min*		
1	7 5	M	15 0	25.0	66.7
2	8.5	F	10 0	23 0	130 0
3	6 9	M	34 0	76 0	123.5
4	7.0	F	8 0	44.0	450.0
5	5 0	M	12.0	47 5	295 8
6	5 0	F	13.0	26 0	92 6
7	5 0	F	11.2	25 0	122 2
8	5 5	F	16 5	27.5	66.7
9	5 3	F	13.2	17.5	32 5
Average	6 3		14.8	34 6	153 3

[4]
$$\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}} \; 3.4$$

where

M_1 = duration of anesthesia of dogs after cholesterol I.P.

M_2 = duration of anesthesia of dogs before cholesterol I P.

ϵ_1 and ϵ_2 = standard errors of cholesterol and control figures respectively.

ANESTHESIA

XXIV. The Effect of Cholesterol on Pentothal and Ether Anesthesia[1]

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Received for publication November 21, 1946.

Starkenstein and Weden (1) showed that intraperitoneal cholesterol injections in rabbits increased the rapidity of onset and the duration of sleep produced by intravenous sodium barbital injections. Beecher and Foldes (2) extended this work, but confined their studies mainly to rabbits, with pentobarbital sleep and sleep from ether injected subcutaneously in the nape of the neck. In these experiments we repeated and confirmed the work of the foregoing investigators in 96 rabbits. Owing to the fact that cholesterol excretion proceeds at a slower rate in carnivorous than in herbivorous animals (3) we included dogs and mice in our studies. Furthermore, as the duration of anesthesia[3] with shorter acting barbiturates is more constant, we chose pentothal sodium for study. In addition, having employed the anesthetic index technic (4) frequently in the dog in the study of new anesthetic agents we endeavored to use this procedure to determine the effect of cholesterol on ether-inhalation anesthesia.

Experimental. *Cholesterol on pentothal anesthesia.* Rabbits of an average weight o 2 kg. were injected intravenously with 0.4 cc./kg. with 2.5 per cent pentothal sodium solution. The onset of sleep was within 10 to 30 seconds. The experimental animals were injected intravenously with 2.5 cc./kg. of a 2 per cent cholesterol suspension in a 25 per cent carbowax solution 30 minutes before the injection of the pentothal sodium.

The effects on the test rabbits and their control groups are shown in table 1 The data show that the suspending agent carbowax had no influence on the duration of pentothal sodium anesthesia, whereas cholesterol, on the average tripled the duration of anesthesia.

Nine mongrel dogs were subjected to essentially the same procedures as described in the foregoing experiment. The same dogs were used in the pentothal and in the pentothal plus cholesterol anesthesia after 7 days. In dogs 40 minutes elapsed after the injection of the

[1] The expense of this investigation was defrayed in part by a grant from the Ohio Chemical and Manufacturing Company, of Cleveland, Ohio.

[2] A portion of a thesis submitted to the Graduate School of the University of Maryland in partial fulfillment of the degree of Master of Science.

[3] Although Beecher and Foldes used the term "sleep" to express the pharmacologic syndrome produced by pentobarbital and ether, we prefer the term anesthesia for our pentothal and ether studies. We used the return of the righting reflex as the endpoint in the pentothal and ether-injection anesthesia. The syndrome could be designated as light surgical anesthesia.

cholesterol before the pentothal was injected. Pentothal sodium was injected intravenously, 0.5 cc./kg. of a 2.5 per cent solution. The cholesterol was injected intraperitoneally, 2.4 cc./kg. of a 12.5 per cent suspension in 6 per cent acacia solution.

The data are given in table 2. They show, when subjected to statistical[4] analysis, that in the dog, as in the rabbit, injections of cholesterol significantly lengthen the anesthesia period with pentothal. From the standard errors of the two series of data the variation observed would occur only once in a thousand trials by chance.

TABLE 1

The effect in rabbits of the intravenous injection of sodium pentothal after the intravenous administration of cholesterol

SUBSTANCE ADMINISTERED	NO. OF RABBITS	AV. WT.	ANESTHESIA DURATION
		kg.	min.
	25	2.14	5.68 ±0.45
2.5 cc./kg. 25% Carbowax..................	15	2.01	5.75 ±0.71
2.5 cc./kg. of a 2% suspension of cholesterol in 25% Carbowax solution................	13	2.00	16.72 ±2.23

TABLE 2

The effect in dogs of the intravenous administration of sodium pentothal before and after the intraperitoneal administration of cholesterol

DOG	WT.	SEX	CONTROL ANESTHESIA	ANESTHESIA (MIN.) AFTER 2.4 CC./KG. OF A 12.5% SUSPENSION OF CHOLESTEROL IN 6% ACACIA	PER CENT CHANGE IN DURATION OF ANESTHESIA OWING TO CHOLESTEROL
	kg.		min.		
1	7.5	M	15.0	25.0	66.7
2	8.5	F	10.0	23.0	130.0
3	6.9	M	34.0	76.0	123.5
4	7.0	F	8.0	44.0	450.0
5	5.0	M	12.0	47.5	295.8
6	5.0	F	13.0	26.0	92.6
7	5.0	F	11.2	25.0	122.2
8	5.5	F	16.5	27.5	66.7
9	5.3	F	13.2	17.5	32.5
Average.......	6.3		14.8	34.6	153.3

[4]
$$\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}\ 3.4$$

where

M_1 = duration of anesthesia of dogs after cholesterol I.P.
M_2 = duration of anesthesia of dogs before cholesterol I.P.
ϵ_1 and ϵ_2 = standard errors of cholesterol and control figures respectively.

Cholesterol on ether anesthesia. We repeated the work of Beecher and Foldes (2) in its essential feature by giving ether to rabbits subcutaneously before and after intraperitoneal injections of cholesterol. The results are shown in table 3. The difference is statistically significant with respect to the duration of anesthesia.[5]

With dogs we employed the anesthetic index technic (4) previously described. In a closed circuit the dog was permitted to inhale 2 cc. of ether and then 0.5 cc./minute until surgical anesthesia was produced. The agent was continued at the same rate until respiratory arrest occurred. The number cc./kg. required to produce respiratory arrest divided by cc./kg. required to produce anesthesia is designated as the anesthetic index. The cholesterol was administered intraperitoneally. Blood-cholesterol levels were determined at several stages of the experiment. No significant relationship could be established between the blood-cholesterol levels and the effect on the duration of the ether anesthesia. The results are shown in table 4.

TABLE 3

The effect in rabbits of the subcutaneous administration of ether after the intraperitoneal injection of cholesterol

SUBSTANCE	NO. OF RABBITS	AV. WT.	ANESTHESIA IN MINUTES	
			Induction	Duration
		kg.		
	39	2.3	9.90 ±0.57	26.34 ±1.47
0.5 gm. cholesterol in 2 *cc.* 6% acacia......................	28	2.2	10.33 ±0.89	38.39 ±2.91

The results in table 4 show that the previous injection of cholesterol intraperitoneally did not significantly reduce the volume of ether required to induce anesthesia or to produce respiratory arrest respectively. The anesthetic index was not significantly changed.

Induction period and cholesterol (mice). We employed the method developed by Knoefel and Murrel (5) to measure the induction time in mice, with and without cholesterol. The method was also used successfully by Lindgren (6) to show the effect of peroxide on the induction time of ethyl ether. A 2.2 liter jar was employed in which a concentration of 2.0 millimoles per liter of ether was maintained. Cholesterol suspension, 2 mg. per animal in 6 per cent acacia solution, was injected intraperitoneally 40 minutes before the anesthetic was administered.

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5 $$\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}\ 3.7$$

M_1 = average duration of anesthesia of cholesterol group.
M_2 = average duration of anesthesia control group.
ϵ_1 and ϵ_2 = standard errors of cholesterol and control groups respectively.

The data from these experiments are shown in table 5. From the analysis[6] of these data it is evident that cholesterol reduces the induction time of ether anesthesia in the mouse.

TABLE 4

The effect of cholesterol on ether anesthesia in dogs

NO.	WT.	SEX	CONTROL ETHER			ETHER AND CHOLESTEROL		
			To induce S.A *	To induce R.F.	A.I.	To induce S.A	To induce R.F.	A.I.
	kg.		*cc /kg*	*cc /kg.*		*cc /kg.*	*cc./kg*	
1	4.60	F	1.36	2.82	2.07	1.09	2.07	2.09
2	7.65	F	1.08	2.77	2.56	1.11	2.35	2.12
3	6.75	M	1.18	2.50	2.13	0.82	2.15	2.64
4	5.00	M	1.14	2.53	2.18	1.10	2.30	2.09
5	5.40	M	1.47	3.53	2.40	1.30	2.78	2.14
6	5.50	M	0.85	2.30	2.70	0.91	2.10	2.40
7	5.00	M	1.20	2.26	1.92	1.10	2.50	2.27
8	5.00	M	0.96	2.85	3.00	0.90	2.10	2.33
9	5.10	F	1.13	2.46	2.16	1.08	2.65	2.45
10	5.20	M	1.23	2.16	1.92	0.87	2.02	2.33
11	5.35	F	1.31	2.62	2.00	1.49	2.99	2.00
12	7.00	F	1.29	2.36	1.83	1.00	2.36	2.36
13	5.20	M	1.07	2.68	2.50	1.06	2.31	2.18
14	8.00	F	1.19	2.80	2.35	1.00	2.63	2.63
15	6.60	F	0.92	2.25	2.46	1.06	2.65	2.50
16	5.60	M	1.13	2 50	2.21	0.89	2.05	2.30
Average.	5.81		1.23	2.59	2.27	1.05	2.38	2.24

* S.A., surgical anesthesia; R.F., respiratory failure; A.I., anesthetic index.

TABLE 5

The effect of cholesterol on the induction time of ethyl ether in mice

SUBSTANCE	NO OF MICE	AV. WT.	INDUCTION
		gm.	*minutes*
	57	15.80	4.77 ±0.38
2 mg. cholesterol in 0.2 cc. 6% acacia 40 minutes before anesthesia	45	15.14	3.20 ±0.07

Discussion. Previously we have pointed out that the syndrome of depression produced by the steroid compounds has been improperly referred to as

[6] $$\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}} \quad 4$$

M_1 is the average induction time of the control group.
M_2 is the average induction time of the experimental group.
ϵ_1 and ϵ_2 are the standard errors of the control and the experimental group, respectively.

Cholesterol on ether anesthesia. We repeated the work of Beecher and Foldes (2) in its essential feature by giving ether to rabbits subcutaneously before and after intraperitoneal injections of cholesterol. The results are shown in table 3. The difference is statistically significant with respect to the duration of anesthesia.[5]

With dogs we employed the anesthetic index technic (4) previously described. In a closed circuit the dog was permitted to inhale 2 cc. of ether and then 0.5 cc./minute until surgical anesthesia was produced. The agent was continued at the same rate until respiratory arrest occurred. The number cc./kg. required to produce respiratory arrest divided by cc./kg. required to produce anesthesia is designated as the anesthetic index. The cholesterol was administered intraperitoneally. Blood-cholesterol levels were determined at several stages of the experiment. No significant relationship could be established between the blood-cholesterol levels and the effect on the duration of the ether anesthesia. The results are shown in table 4.

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$$\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}\ 3.7$$

M_1 = average duration of anesthesia of cholesterol group.
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TABLE 4

The effect of cholesterol on ether anesthesia in dogs

NO	WT.	SEX	CONTROL ETHER			ETHER AND CHOLESTEROL		
			To induce S.A.*	To induce R.F.	A.I.	To induce S.A.	To induce R.F.	A.I.
	kg		*cc/kg.*	*cc/kg.*		*cc/kg.*	*cc/kg*	
1	4.60	F	1.36	2.82	2.07	1.09	2.07	2.09
2	7.65	F	1.08	2.77	2.56	1.11	2.35	2.12
3	6.75	M	1.18	2.50	2.13	0.82	2.15	2.64
4	5.00	M	1.14	2.53	2.18	1.10	2.30	2.09
5	5.40	M	1.47	3.53	2.40	1.30	2.78	2.14
6	5.50	M	0.85	2.30	2.70	0.91	2.10	2.40
7	5.00	M	1.20	2.26	1.92	1.10	2.50	2.27
8	5.00	M	0.96	2.85	3.00	0.90	2.10	2.33
9	5.10	F	1.13	2.46	2.16	1.08	2.65	2.45
10	5.20	M	1.23	2.16	1.92	0.87	2.02	2.33
11	5.35	F	1.31	2.62	2.00	1.49	2.99	2.00
12	7.00	F	1.29	2.36	1.83	1.00	2.36	2.36
13	5.20	M	1.07	2.68	2.50	1.06	2.31	2.18
14	8.00	F	1.19	2.80	2.35	1.00	2.63	2.63
15	6.60	F	0.92	2.25	2.46	1.06	2.65	2.50
16	5.60	M	1.13	2.50	2.21	0.89	2.05	2.30
Average..	5.81		1.23	2.59	2.27	1.05	2.38	2.24

* S.A., surgical anesthesia; R.F., respiratory failure; A.I., anesthetic index.

TABLE 5

The effect of cholesterol on the induction time of ethyl ether in mice

SUBSTANCE	NO. OF MICE	AV. WT.	INDUCTION
		gm	*minutes*
	57	15.80	4.77 ±0.38
2 mg. cholesterol in 0.2 cc. 6% acacia 40 minutes before anesthesia	45	15.14	3.20 ±0.07

Discussion. Previously we have pointed out that the syndrome of depression produced by the steroid compounds has been improperly referred to as

[6] $$t = \frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$$

M_1 is the average induction time of the control group.
M_2 is the average induction time of the experimental group.
ϵ_1 and ϵ_2 are the standard errors of the control and the experimental group, respectively.

"anesthesia" (7). It is reasonable to assume that any compound which elicits a depression of the central nervous system would exert an additive effect or potentiation of the action of a general anesthetic. The mechanism of action of cholesterol in these studies may be explained on such a basis. Our observation on dogs and rabbits revealed depression produced by intraperitoneal and intravenous cholesterol injections.

Cholesterol increased the potency of ether in the mouse upon inhalation, but not in the dog. It is possible that this is due to a species variation, but we believe that if cholesterol produced only a slight effect, the anesthetic index procedure would not detect it, owing to the lack of precision inherent to the experiment.

We have succeeded in lengthening the period of pentothal anesthesia in the rabbit and dog by previous intravenous or intraperitoneal injections of cholesterol. We were unable to obtain a significant correlation between cholesterol-blood levels and the effect of this sterol upon anesthesia in the dog. Perhaps it is the concentration of cholesterol in the tissue of the central nervous system which is the dominating factor in its effect upon the anesthetic syndrome. In addition, cholesterol may exert an accelerating effect upon the absorption of ether from subcutaneous depots.

SUMMARY

1. The intravenous or intraperitoneal injection of cholesterol in the rabbit and the dog has been shown to intensify the anesthetic action of pentothal sodium.

2. Cholesterol injections lengthened the anesthetic syndrome in the rabbit produced by subcutaneous injections of ether.

3. Cholesterol injections produced no significant effect upon the anesthetic syndrome in the dog, using the inhalation technic.

4. Cholesterol injections diminished the ether induction period in mice.

REFERENCES

1. STARKENSTEIN, E., AND WEDEN, H.: Arch. f. Exp. Path. u. Pharmakol., 182: 700, 1936.
2. BEECHER, H. K., AND FOLDES, F. F.: THIS JOURNAL, 78: 276, 1943.
3. CASHIN, M. F., AND MORAVEK, V.: Am. J. Physiol., 82: 294, 1927.
4. KRANTZ, J. C., JR., CARR, C. J., FORMAN, S., AND EVANS, W. E., JR.: THIS JOURNAL, 69: 207, 1940.
5. KNOEFFEL, P. K., AND MURRELL, F. C.: THIS JOURNAL, 55: 235, 1935.
6. LINDGREN, E.: Svensk farm. tidskr., 47: 17, 1943.
7. FARSON, D. B., CARR, C. J., AND KRANTZ, J. C., JR.: Proc. Soc. Exp. Biol. and Med., 63: 70, 1946.

A TOXICOLOGIC STUDY OF TWO HISTAMINE ANTAGONISTS OF THE BENZHYDRYL ALKAMINE ETHER GROUP

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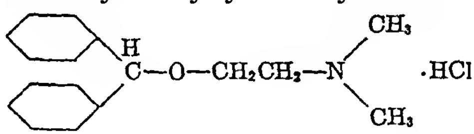
Received for publication November 27, 1946

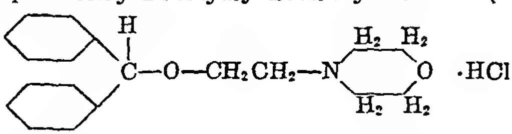
Using modified Schaumann (1) and Halpern (2) methods for inducing histamine shock in guinea pigs, Loew, Kaiser and Moore (3), in their study of benzhydryl alkamine ethers, found that two substances, β-dimethylaminoethyl benzhydryl ether (Benadryl) and β-morpholinoethyl benzhydryl ether (A-446), were effective in protecting guinea pigs exposed to lethal amounts of histamine vapors. The ratio of the protective activity of the two compounds was roughly 2 to 1 in favor of Benadryl. Preliminary studies indicated that toxicity of Benadryl was greater than that of compound A-446 (3, 4). Analysis of data accumulated since 1943 on both compounds is presented herewith.

CHEMICAL AND PHYSICAL PROPERTIES. β-Dimethylaminoethyl benzhydryl ether hydrochloride differs from β-morpholinoethyl benzhydryl·ether hydrochloride in substitution of the dimethyl amino group by the morpholino radical as shown by their structural formulae.

β-Dimethylaminoethyl Benzhydryl Ether Hydrochloride (Benadryl)



β-Morpholinoethyl Benzhydryl Ether Hydrochloride (A-446)



Both Benadryl and A-446, are white crystalline substances, soluble in water and alcohol. The aqueous solutions are stable under ordinary conditions of temperature and light. The 1.0 per cent aqueous solutions have a pH reaction of about 5.0. Both compounds possess a bitter taste.

ACUTE TOXICITY. Normal animals under standard conditions were given Benadryl and A-446 by cannula orally, intravenously, subcutaneously and intraperitoneally. The animals were observed for 7 to 14 days. Results were evaluated by the double integration method of Dragstedt (5).

With the exception of intravenous toxicity in rats, the morpholinoethyl benzhydryl ether was about one-half as toxic as the dimethylaminoethyl benzhydryl ether. Death following administration of either compound appeared to

be due to a combined effect of violent excitement, convulsive attacks, prostration and respiratory and cardiac failure. If death did not occur during the violent excitement following the administration, the animals recovered quickly and remained normal indefinitely. An intravenous dose of 5 mg./kg. of A-446 produced no reactions in dogs, the same dose of Benadryl caused some apprehension and barely noticeable spasticity of the legs. A dose of 10 mg./kg. of Benadryl produced a state of panic, spasticity, apprehension, and occasionally the animal developed a clonic convulsive attack of short duration. It took about 15 mg./kg. of A-446 to cause similar degree of reactions. A dosage of 20 mg./kg. of either compound caused violent excitement, prostration, and convulsions. Animals became sensitive to sound, and developed painful sensation of the skin

TABLE 1

Comparative results in acute toxicity of benzhydryl alkamine ethers

ROUTE	BENADRYL			A-446		
	Number of animals	Mg./kg.		Number of animals	Mg./kg	
		LD-0	LD-50		LD-0	LD-50
Albino mice						
Oral	1060	50.0	164.0	670	175.0	327.0
Subcutaneous	740	50.0	127.0	640	100.0	440.0
Intraperitoneal	270	37.5	98.0	225	75.0	185.0
Albino rats						
Oral	485	100.0	500.0	155	200	916.0
Subcutaneous	230	200.0	474.0	—	—	—
Intravenous	1124	20 0	42.0	253	253	35.0
Rabbits						
Intravenous	25	7.5	10 0	29	17.5	21.0
Dogs						
Intravenous	42	20 0	24.0	12	60.0	70.0

and feet. Dogs would bite at different parts of the body and feet in rapid succession. Rats chewed off feet. Somatic reflexes became somewhat exaggerated. Pupils became dilated.

Sedation with barbiturates 30 to 90 minutes before intravenous injection of either Benadryl or A-446 prevented excitement and convulsions, but did not affect the mortality rate or lessen the respiratory-cardiac depression.

The reactions in smaller animals were similar in form irrespective of the mode of administration. In mice and rats the reactions were in evidence in about 3 minutes following intraperitoneal administration and in about 5 to 20 minutes for subcutaneous and oral administration. Perorally in dogs, reactions appeared in about 30 to 60 minutes; reached the maximum in another hour and subsided

in the next 1 to 4 hours. On equal doses perorally Benadryl-treated animals became symptom-free more slowly (3 to 6 hours) than the A-446 treated animals (2 to 4 hours).

Single daily injections of 5 mg./kg. of Benadryl to dogs intravenously produced minimal reactions after the first dosing. With subsequent injections the animals appeared apprehensive and scared. In some animals the reactions were more intense than in others. Reactions subsided in 5 to 10 minutes. Compound A-446 caused no reactions on repeated intravenous injections of 5.0 mg./kg. doses. Administration of 10 mg./kg. of either compound caused considerable excitement, fear of sound, and spasticity of the legs, and an animal occasionally developed a single convulsive attack. With subsequent injections the reactions tended to become less intense, but no tolerance developed in 12 days. The animals became symptom-free in about 20 to 30 minutes.

TABLE 2

Benzhydryl alkamine ether ingestion by albino mice for 14 days. Growth rate and mortality

	PER CENT SUBSTANCE IN FOOD					CONTROLS
	0.05	0.10	0.25	0.5	1.0	
A-446						
Mg./kg./day	93	185	455	631	912	0.0
Weight gain, grams/M.	2.9	0.8	1.2	*-.30	-.40	2.3
Mortality, per cent	0	0	0	32	100	0.0
	LD-50 = 717 mg./kg.					
Benadryl						
Mg./kg./day	100	182.5	469	540	828	0.0
Weight gain, grams/M.	0.8	0.4	*-2.6	-5.0	-5.0	2.3
Mortality, per cent	0.0	4.0	20	100	100	0.0
	LD-50 = 493 mg./kg.					

* — = loss in weight per mouse.

Chronic toxicity. Albino mice in groups of 25 animals each were placed on basic diet containing different concentrations of benzhydryl alkamine ether compounds. The food consumption was measured daily for each 24 hour period. The animals were weighed in groups at weekly intervals, and the mean daily drug intake in each group was calculated as shown in table 2.

The daily ingestion of A-446 caused no death in mice at 455 mg./kg. while one mouse out of 25 died on 182.5 mg./kg. Benadryl. Mice gained in weight normally on 93 mg./kg. per day of A-446, while 100 mg./kg. of Benadryl depressed the growth rate. The weight depression by 100 mg./kg. per day of Benadryl was about equivalent to that of 185 mg./kg. of A-446. On the basis of chronic toxicity studies the LD-50 for A-446 in mice was equal to 717 mg./kg. per day and for Benadryl 493 mg./kg.

Albino male rats of average weight of 90-109 gms. in groups of 20 animals each

were placed on Benadryl food-diet for a period of 28 days. The amount of food ingested was measured daily for the whole group of animals, and weights of individual animals were recorded at weekly intervals. The Benadryl intake was estimated from the average daily food ingestion for the whole period. Results in the form of averages are summarized in table 3.

Benadryl ingestion by rats resulted in normal or over normal gain in weight on an average intake of 72 to 157.6 mg./kg. per day. Ingestion of 249 mg./kg. depressed gain in weight and caused a *10* per cent mortality. The *LD-50* mortality occurred with ingestion of about 266 mg./kg. per day of Benadryl.

Tolerance in dogs perorally. Normal adult dogs were given Benadryl and A-446 daily for periods of 18 days to over six months. The compounds were given in gelatin capsule to some animals in two divided doses and in others in a single daily dose about two to six hours before feeding. Amounts of 10, 25, 60, and 80 mg./kg. were given to respective groups of 3 to 5 animals each.

TABLE 3

Benadryl tolerance in albino rats. Growth rate and mortality

	BENADRYL PER CENT IN FOOD						CONTROLS
	0.075	0.10	0.125	0.25	0.5	1.0	
Benadryl, mg./kg./day	72	101	157.6	249	719*	—†	0
Gain, gms./rat in 28 days	141	122	79	22	−10	—	68
Mortality, per cent	0	0	0	10	85	100	0
	LD 50 = 266 mg./kg.						

* The results unreliable, rats were wasting food.

† Animals became sick-looking inside of 48 hours, thereafter refused food and all were dead inside of the first seven days.

Benadryl caused no reactions in doses of 10 mg./kg. in two divided or in a single daily dose to five dogs for periods of 18, 145, 183, 193, and 193 days respectively. The A-446 compound was well tolerated in doses of 10 to 25 mg./kg. for 35 to 48 days, except for an occasional emesis about one hour after feeding. Neither compound, in the above doses, produced disturbance in hematology or total blood non-protein nitrogen. The urine remained free of albumen and sugar. The animal weight fluctuated in a narrow range of ±0.5 kilogram.

A dosage of 25 to 40 mg./kg. of Benadryl in two divided daily doses for a period of 183 to 196 days caused some alertness and an occasional emesis. When the same amounts were given in a single daily dose the animals became irritable, excited, developed spasticity in the extremities and slight incoordination. The single daily dosage of 40 mg./kg. in addition caused painful skin paraesthesia. The reactions appeared in about 1 to 2 hours after dosing and lasted for about 2 to 4 hours. These symptoms occurred during the first five days of treatment with 25 mg./kg., thereafter the animals appeared more alert, somewhat sensitive to sudden loud sounds, but otherwise reactions were absent, while with 40 mg./kg. in a single daily dose the reactions recurred with each dosing for the whole

treatment period of 36 days. No cumulative aggravation of reactions was apparent.

A single daily dosage of 60 mg./kg. caused severe spastic ataxia, excitement, hypersensitivity to loud sounds, and a painful skin sensation. Salivation developed in some animals in the third week of dosing and thereafter became a conditioned reflex on disturbance of the animal.

In comparison, compound A-446 in a single daily dose of 40 mg./kg. caused no reactions, except slight lowering of the red cell count and hemoglobin values. A dosage of 60 mg./kg. produced occasionally spastic ataxia, excitement and irritability to loud sounds. A dosage of 80 mg./kg. of A-446 in about half to one hour after dosing produced severe incoordination, excitement, irritability, tremors, spastic ataxia and a severe general painful hyperaesthesia. The animals recovered in about 6 to 8 hours. No cumulative aggravation of reactions occurred in a period of 35 days. There was, however, a considerable loss in weight (1.5 kgs.), probably due to persistent anorexia. The animals developed mild albuminurea. The red cell counts and hemoglobin values decreased slightly. The total blood non-protein nitrogen remained undisturbed.

It was apparent that Benadryl was tolerated in 25 mg./kg. in two divided daily doses. A single daily dose of 25 to 40 mg./kg. caused considerable neurogenic reactions. A-446 in a single daily dose showed no reactions until a dosage of 60 mg./kg. was reached, comparable to a single daily dose of 25 mg./kg. of Benadryl. The intensity of reactions from a single daily dose of 40 mg./kg. of Benadryl was about equivalent to 80 mg./kg. of A-446. The action of Benadryl was somewhat more prompt and lasted somewhat longer than that of A-446. The latter compound had a tendency to cause anemia and a mild albuminurea.

Local tissue irritation. In mice, rats and guinea pigs subcutaneous and intramuscular injections of 0.1 to 1.0 cc. of one per cent concentrations of Benadryl caused induration and ulceration at the site of injection. Intramuscular injections caused more severe local tissue injury than subcutaneous doses. In rabbits, subcutaneous injection of 0.5 to 2 cc. of 1.0 per cent solution produced punctate hemorrhages in the fascia, but otherwise the injury was slight. Intramuscular injection of 0.5 to 4.0 cc. caused severe induration and necrosis. In dogs, 0.5 to 3.0 cc. of one per cent concentration subcutaneously caused slight hyperemia, some local induration and mild ecchymosis. Intramuscularly, 0.5 to 2.0 cc. dosage caused slight congestion, but no induration or necrosis of tissues was present. A dose of 6 cc. caused a small area of necrosis with some induration. Absorption was rapid.

Allergy studies in guinea pigs. Three groups of twelve guinea pigs each received respectively daily subcutaneous injections for 21 days of 2.5, 5.0 and 7.5 mg./kg. of Benadryl. In addition to loss in weight, all pigs developed local tissue induration which was occasionally followed by ulceration in some pigs.

The pigs were rested for 37 days and then were injected subcutaneously or intraperitoneally with 5 to 10 mg./kg. of Benadryl. None of the pigs showed reactions immediately or subsequently. It was evident that Benadryl did not sensitize guinea pigs and no anaphylactic reactions developed.

PHARMACODYNAMICS. In the anesthetized dog, toxic doses of Benadryl intravenously and perorally produced blood pressure depression. The heart rate was accelerated in small doses and retarded on larger doses. Respiration was stimulated by small doses and became restricted in depth with larger doses, followed by respiratory failure, placid prostration and death.

In unanesthetized dogs on toxic doses, after an initial increase in rate, the respiration became shallow, irregular, slow, and labored, followed by respiratory failure. The pulse became fast and weak at first, followed by strengthening and slowing. After respiratory failure, the pulse became rapid and feeble, and then ceased. On the contrary, if the respiration did not fail the respiration rate increased and the pulse rate became slower and stronger and the animal recovered.

In small doses the pulse rate remained unchanged or became slightly depressed. The respiratory rate was stimulated.

PATHOLOGY. The early histopathologic changes in animals dying were confined to congestion and edema of the lungs and congestion of visceral organs, liver, kidneys, spleen, adrenals and gastro-intestinal tract mucosa. Animals which survived the acute effect of Benadryl or A-446, after a rest period of 48 hours to nine days, displayed no pathologic changes on necropsy and on microscopic examination of tissues.

Animals given multiple parenteral non-lethal doses, on necropsy and on microscopic examination of the visceral organs and brain tissues, were free of degenerative tissue changes.

Peroral administration of single toxic doses to smaller animals, (mice and rats), developed acute symptoms of toxicity and death within two hours accompanied by prostration and respiratory failure. Pathologic changes were similar to those from parenteral administration: severe congestion of the lungs and visceral organs. A prolonged ingestion by mice of 100 to 185 mg./kg. of A-446 and Benadryl daily by mice and 249 mg./kg. of Benadryl by rats, caused gradual loss in weight and cachexia. Histologic study showed moderate to severe chronic inflammatory foci in lungs, congestion of spleen, and slight edema of liver with mild spotty fatty degenerative infiltration. The kidneys, adrenals, pancreas, and brain tissues appeared normal. The intestinal tract was considerably dilated and atonic, but no acute inflammation or necrosis was found. The thyroid glands showed mild depletion of colloid substance. The acinal cells showed mild hypertrophy.

Histologic material of dogs ingesting Benadryl or A-446 in doses of 10 mg./kg. in single or divided daily doses revealed no cumulative tissue degeneration. The kidneys and urinary bladder were free of calculi. The gastro-intestinal mucosa on dose levels up to 25 mg./kg. appeared normal. Dosage of 40 to 80 mg./kg. caused mild congestion and scattered petechial hemorrhages of the gastro-intestinal mucosa, particularly in the case of A-446.

Clinical and histopathologic evidence indicates that death in animals following administration of lethal doses of Benadryl and A-446 was due to a violent neuromotor excitement, convulsions, respiratory failure and myocardial depression, accompanied by congestion of visceral organs and anoxia. Death on prolonged

ingestion by small animals of the respective compounds was due to combined effect of cachexia, gastro-intestinal atony, congestive stasis of visceral organs and superimposed inflammatory foci in lungs. In larger animals, (dogs), no parenchymatous tissue changes occurred on ingestion of 10 to 80 mg./kg.

SUMMARY

Two compounds, β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl), and β-morpholinoethyl benzhydryl ether hydrochloride (A-446), possessed in experimental animals pharmacologic properties similar in type, but different in intensity. The intensity of reactions and toxicity of Benadryl was roughly twice that of the morpholino compound.

In aminals both substances caused a complex syndrome of excitant reactions predominantly neurogenic in origin involving motor, sensory and autonomic nervous systems. Small doses increased the cardiac and respiratory rates.

Irrespective of the mode of administration, toxic doses of either compound caused excitement, spastic ataxia, extreme irritability, sensitivity to sound, mydriasis, painful hyperaesthesia, convulsive attacks, and respiratory and myocardial embarrassment. Death occurred from respiratory and myocardial depression following violent excitement and terminal prostration.

Duration of reactions varied with the route of administration. Dogs recovered in 15 minutes to about 3 hours from intravenous and 2 to 8 hours from peroral administration of non-lethal amounts.

Intravenously to dogs 2.5 mg./kg. of Benadryl and 5.0 mg./kg. of A-446 caused no reaction. Perorally 12.5 to 20 mg./kg. of Benadryl, and 20 to 30 mg./kg. of A-446 twice daily were well tolerated. Neither Benadryl or A-446 caused somnolence or cumulative toxic action in animals. No tolerance, sensitization or anaphylactic reactions occurred.

The histopathologic changes produced by toxic doses of both compounds were related to vasodilation, congesticn and stasis. In tolerated doses no acute or cumulative degenerative tissue changes occurred. The hematologic picture and physiologic function of liver and kidneys were essentially unaffected by either compound.

Barbiturates controlled excitant neurologic reactions, but did not prevent respiratory-cardiac depression.

Benadryl and A-446 were well tolerated in animals in appropriate dosage range for a period of six months. The former was the more active compound and on the basis of this study was submitted to clinical trials, results of which have been reported elsewhere.

BIBLIOGRAPHY

1. Schaumann, O.: Arch. exp. Path. u. Pharmacol., 109: 196, 1940.
2. Halpern, B. N.: Arch. internat. de pharmacodyn. et de Therap., 68: 339, 1942.
3. Loew, E. R., Kaiser, M. E., and Moore, V.: This Journal, 83: 120, 1945.
4. Rieveschl, G. and Gruhzit, O. M.: Federation Proc., 4: 150, 1945.
5. Dragstedt, C. A. and Lang, V. T.: This Journal, 32: 215, 1927-1928.

COMPARATIVE ACTIVITY OF VARIOUS ANTIHISTAMINIC SUBSTANCES TOWARDS THE VASODEPRESSOR RESPONSE TO HISTAMINE

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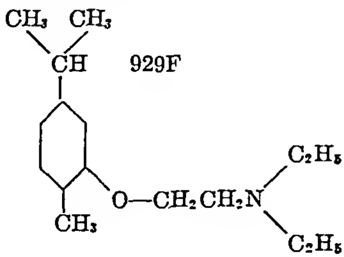
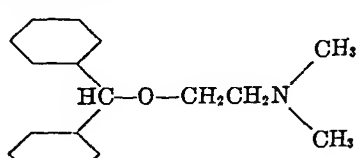
Received for publication December 14, 1946

In 1937, Bovet and Staub (1) reported that 929F[1] had the ability to prevent the lethal effects of histamine intravenously administered in guinea pigs, and later Staub (2) extended this work to include other Fourneau compounds, of which 1571F was apparently the most active. In 1942, Halpern (3) indicated that considerably more active compounds were possible, of which 2339RP was the most active and least toxic. Recently Loew and coworkers (4) have reported "Benadryl" as the most active of a series of homologous ethers related to 929F, and Mayer, Huttrer and Scholz (5) have reported 63C, or "Pyribenzamine", as the most active member of a series of pyridine analogs of 1571F and 2339RP. It is well known that atropine is able to antagonize certain histamine effects on isolated segments of intestine and a great volume of work has been done with related, synthetic esters in the search for antispasmodic agents. Lands, Nash and Hooper (6) reported several anticholinergic esters that had considerable antihistamine activity. Since the reports concerning many of these compounds appeared in the literature almost simultaneously, no one has investigated them on a comparative basis. Experiments herein reported were conducted to determine the relative antagonism of these various compounds for the transient fall in blood pressure produced by the intravenous administration of histamine. Wells and coworkers (7) have assayed the activity of Benadryl by comparing the amounts of histamine required to produce a given fall in blood pressure before and after Benadryl, while Loew (4) compared the reduction in depressor response produced by a repeated small dose of histamine. Both of these procedures have been extended to cover the compounds under investigation whose structural formulas are given below:

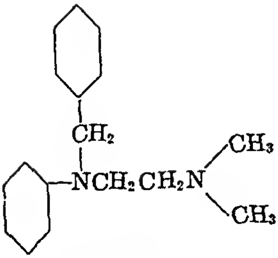


929F

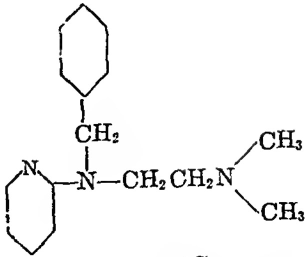


S51
Benadryl

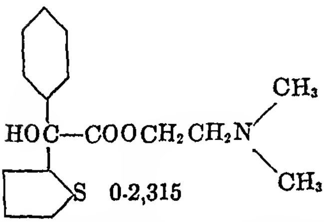
[1] The compounds under investigation in this paper are referred to by the trivial reference numbers used by other workers or by trade names since no accepted, common or official names are available and the proper chemical names are cumbersome.



2339RP
Antergan



63C
Pyribenzamine



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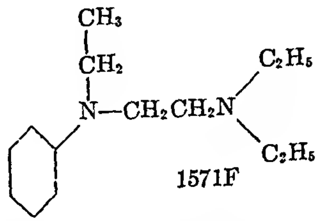

1571F

EXPERIMENTAL. *A. Experiments in dogs.* The intravenous injection of small amounts of histamine elicits a transient fall in blood pressure in the dog that, under conditions of uniform anesthesia and constant level blood pressure, is quantitatively reproducible. This depressor response is alleged to be due to relaxation of arteriolar smooth muscle and capillaries (8).

PROCEDURE. Experiments were made on thirty apparently healthy mongrel dogs (5-12 kgm.) anesthetized with 350 mgm./kgm. sodium barbital administered intraperitoneally ninety minutes prior to operation. Blood pressure was recorded by an ink-writing mercury manometer connected to the carotid artery. The majority of these dogs were vagotomized to prevent reflex interference with the epinephrine response. All injections were made into an exposed femoral vein. In each experiment, test doses of one and two micrograms/kgm. of epinephrine were given, and then 1, 2, 5, and 10 micrograms/kgm. of histamine (equivalent to 3, 6.5, 16, and 32 $\times$ 10^{-9} Moles/kgm.) acid phosphate, 5 $\times$ 10^{-6} moles/kgm. of the histamine antagonist, and the epinephrine and histamine repeated. An additional 1.0 $\times$ 10^{-5} Moles/kgm. of the agent was administered, and the epinephrine and histamine again repeated. Where it was possible to give larger doses of the agent without producing a permanent reduction in the blood pressure, 3.5 $\times$ 10^{-5} Moles/kgm. (total dose 5 $\times$ 10^{-5} Moles/kgm.) was given, the epinephrine and histamine being repeated as before except that the dosage of histamine was increased to include the 100 to 1000 microgram/kgm. range.

RESULTS. A cross-section of the results is given in table 1, treated by the method of Loew (4b). Table 2 indicates the histamine-equivalence results following the procedure of Wells (7b).

Staub (2) was unable to produce antidepressor effects for 929F against histamine. Climenko, Homburger, and Messer (9) indicated the relative lack of activity of 929F when tested by a similar procedure. Their results are also considerably complicated by the continuing fall in blood pressure produced by large doses of the agent. In attempts to obviate this undesirable side action,

they administered the 929F subcutaneously in divided doses, but the histamine antagonism is scarcely any better, and the animal still deteriorates fairly rapidly. This same difficulty is encountered with 1571F. Yonkman (10) in a preliminary report on the effects of 63C mentioned that small doses of the agent did not antagonize the histamine vascular effects. However, Sherrod, Schloemer, and Loew (11) reported that considerable histamine antagonism can be produced with larger doses of Pyribenzamine. Their preliminary report did not give any quantitative data, but the results in table 1 are certainly confirmatory. Antergan or 2339RP is effectively able to antagonize the histamine fall in blood

TABLE 1

Per cent decrease in blood pressure fall produced by histamine after antagonist

NAME OF DRUG	AGENT DOSE	HISTAMINE 2 MCG/KGM	AGENT DOSE	HISTAMINE 5 MCG/KGM.	AGENT DOSE	HISTAMINE 10 MCG/KGM
	mgm	%	*mgm.*	%	*mgm.*	%
Thymoxyethyldiethylamine HCl (929F)	1.6	43	4.4	42	16	21
Diethylaminoethyl ethylaniline HCl (1571F)	1.3	−10	3.9	6	12.8[a]	
Dimethylaminoethyl benzylaniline HCl (97B or 2339RP)	1.5	53	4.4	70	14.6	78
Dimethylaminoethyl benzylpyridine HCl (63C)	1.5	80	4.4	55	14.6[b]	
Benzhydryloxyethyldimethylamine HCl (A524 or S51)	1.5	90	4.4	67	14.5	74
Dimethylaminoethyl phenyl-α-thienylacetate HCl (0-2, 327)	1.6	27	4.8	40	16.3[a]	
Dimethylaminoethyl phenyl-α-thienylglycolate HCl (0-2, 315)	1.7	86	5.1	82	17.0	88
Diethylaminoethyl phenyl-α-thienylacetate HBr (606)	2.0	29	6.0	27	19.9[a]	
Diethylaminoethyl phenyl-α-thienylglycolate HCl (600)	1.9	64	5.7	81	18.6[a]	
Piperidinoethyl phenyl-α-thienylglycolate HCl (609)	1.9	95	5.7	46	19.0[a]	

Each per cent figure is the average of the results in three dogs. [a] = above the immediate LD50. [b] = prolonged fall in blood pressure.

pressure. Halpern did some preliminary experiments in which five milligrams of Antergan gave about fifty per cent reduction in the fall produced by twenty-five micrograms of histamine. The results with Benadryl are similar to those of Loew (4b) except that slightly better antagonism has been obtained than he reported, and in this respect, the results are more comparable to Wells (7b). Also, we found that if the dose of Benadryl is adequately increased, further reduction in histamine effect is possible, which is partly in disagreement with Loew. In the histamine-equivalence method of Wells, we found it best if doses of ten micrograms/kgm. of histamine were not exceeded early in the

experiment, as larger doses of histamine apparently left the animal in poor condition so that the administration of 14.5 or 29 mgm./kgm. of Benadryl promptly produced peripheral vasomotor collapse from which the animal did not recover. Barbitalized dogs that had received no histamine survived 56 mgm./kgm. of Benadryl without a significant reduction in blood pressure, other than the transient fall immediately following the injection. This apparent potentiation of the deleterious effects of these agents by the prior administration

TABLE 2

Amount of histamine necessary after antagonist to reproduce effects of 5 micrograms of histamine

NAME OF DRUG	AGENT DOSE	HISTAMINE EQUIVALENT	AGENT DOSE	HISTAMINE EQUIVALENT	AGENT DOSE	HISTAMINE EQUIVALENT
	mgm	*mcgm*	*mgm*	*mcgm*	*mgm*	*mcgm*
Thymoxyethyldiethylamine HCl (929F)	1.6	—	4.4	12		
Diethylaminoethyl ethylaniline HCl (1571F)	1.3	—	3 9	8		
Dimethylaminoethyl benzylaniline HCl (97B or 2339RP)	1 5	20	4 4	80	14 6	200
Dimethylaminoethyl benzylpyridine HCl (63C)	1 5	10	4 4	40		
Benzhydryloxyethyldimethylamine HCl (A524 or S51)	1 5	50	4 4	125	14 5	250
Dimethylaminoethyl phenyl-α-thienylacetate HCl (0-2, 327)	1 6	10	4 8	12		
Dimethylaminoethyl phenyl-α-thienylglycolate HCl (0-2, 315)	1 7	25	5 1	100	16 0	500
Diethylaminoethyl phenyl-α-thienylacetate HBr (606)	2 0	10	6 0	10		
Diethylaminoethyl phenyl-α-thienylglycolate HCl (600)	1 9	20	5 7	50		
Piperidinoethyl phenyl-α-thienylglycolate HCl (609)	1 9	15	5 7	40		

The histamine equivalent is the median result of determinations in three dogs for each compound

of large doses of histamine was most noticeable with 929F and 1571F although it did also occur with 63C and 2339RP.

Of the compounds of the type investigated by Lands, Nash and Hooper (6), the dimethylaminoethyl phenyl-α-thienylglycolate (0-2, 315) was the most active, with the other glycolates showing some activity and the acetates very little The differences in the histamine equivalents after the administration of 5×10^{-5} Moles/kgm of Benadryl, Antergan and 0-2, 315 appear quite large, but the actual differences are not great. With large doses of these potent antihistaminic agents, it is often difficult to tell the difference between 0.1 and 1.0 milligrams/kgm of histamine, and if the results are expressed as "per cent"

as Wells (7b) has done, they simply indicate that the animal has been protected against something over 95% of the administered histamine. However, with lower doses and with the less active agents, it is quite easy to measure the difference between two doses of histamine that differ by only one or two micrograms/kgm.

Since the 0–2, 315 is relatively non-toxic and has very little epinephrine potentiating action or other undesirable side-effects, it was considered worthwhile to compare it with Benadryl and Pyribenzamine in other species of animals.

B. Experiments with rabbits. Although Ramanamanjary (12) has published that 929F, 1571F, and 2339RP are inactive when administered subcutaneously to rabbits that have been anesthetized with chloralose, barbiturates, or urethane, it was decided to include rabbits in the investigation by virtue of their normally biphasic blood pressure response to histamine.

PROCEDURE. Experiments were made on seven rabbits anesthetized with 350 mgm./kgm. sodium barbital administered intraperitoneally ninety minutes prior to operation. Three additional rabbits were anesthetized with two grams/kgm. urethane administred intraperitoneally as a warm, twenty-five per cent solution two hours prior to operation. Hamilton hypodermic manometers as modified by Shuler (13) were used to record blood pressure changes. The median circumflex branch of the femoral artery was cannulated with a $\frac{1}{4}$ inch, 24 gauge, cannula so that the tip of the cannula was flush with the lumen of the femoral artery or barely protruding into the femoral artery in order that information concerning the peripheral standing waves and run-off could be obtained (14). Injections were made into the exposed femoral vein of the opposite leg. Twenty micrograms/kgm. of epinephrine and fifty and two hundred micrograms/kgm. of histamine phosphate were injected as test doses, then 0.5 and 1.0 $\times 10^{-5}$ Moles /kgm. of the antihistaminic agent, and the test doses repeated with an additional dose of 1000 micrograms/kgm. of histamine.

RESULTS. Woodbury (15) has indicated that the rabbit may respond to histamine by either vasoconstriction with an accompanying rise in blood pressure or by vasodilation with the usual fall in blood pressure. This phenomenon was observed in our rabbits. 0.5 $\times 10^{-5}$ Moles/kgm. Pyribenzamine, Benadryl, and 0–2, 315 completely antagonized the slight rise produced by fifty or two hundred micrograms of histamine and from forty to sixty per cent of the fall. A total dose of 1.5 $\times 10^{-5}$ M/kgm. (about 5 mgm./kgm.) of these agents antagonized seventy to one hundred per cent of the fall produced by fifty micrograms histamine, sixty to seventy per cent of the fall from two hundred micrograms, and thirty-five to forty-five per cent of the fall from one thousand micrograms. As far as the quantitative results were concerned, it would be difficult to differentiate one of these compounds from the other at these dose levels. None of these compounds produced the transient vasoconstriction sometimes seen in dogs (cf. Sherrod, 11, and Abreu, 16). They all produced vasodilation as evidenced by the loss of the standing-wave from the arterial pulse contour and the more rapid rate of descent of the run-off curve (14). The larger doses also produced considerable myocardial damage as evidenced by the rounding of the systolic peaks. This is understandable, for the LD_{50} for the rabbit for Benadryl is about twice this dose or 11 mgm./kgm. (Rieveschl, 17). Sample sections of a typical experiment are shown in figure 1.

This particular rabbit had a very high systemic arterial pressure and responded to histamine by vasodilation only. The broadening of the pulse ten seconds after the administration of the histamine is only evidence of increased cardiac effort to maintain the blood pressure in the face of the tremendously increased peripheral run-off. This record may be contrasted with that in figure 5 in which the rabbit exhibited systemic vasoconstriction to the test dose of histamine.

C. Experiments with rats and guinea-pigs. The general practice of determining the lethal dose ranges for a drug in the albino rat and the desirable activity in dogs or cats often leads to irrational conclusions concerning effectiveness in relation to safeness. Since some toxicity data for these compounds has been

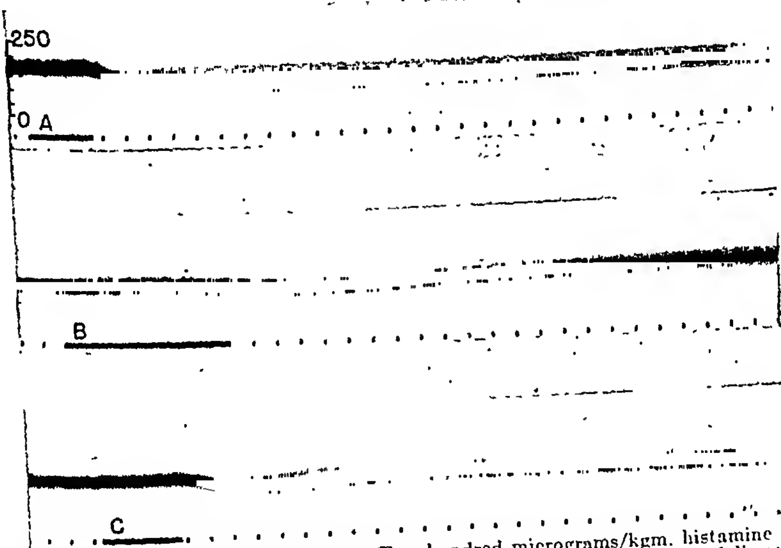

FIG. 1. 3.1 kgm. white male rabbit. Two hundred micrograms/kgm. histamine acid phosphate given intravenously at duration signal A and C. 1 7 mgm /kgm. of dimethylaminoethyl phenyl-α-thienylglycolate HCl (0-2,315) given intravenously at B. Injections given five minutes apart. Time signals 2 5 seconds.

compiled for rats, it was considered of interest to investigate the antihistaminic activity in rats.

PROCEDURE. Six litter-mates were anesthetized with 300 mgm./kgm. sodium barbital administered intraperitoneally. Systolic and diastolic blood pressure was recorded with a Hamilton manometer connected to a cannula in the carotid artery (13). All injections were made into an exposed external jugular vein. In each experiment, one milligram/kgm. of histamine phosphate was injected and after the blood pressure had returned to normal, 1.0×10^{-4} Moles/kgm. of the antihistaminic and after the transient fall in pressure, a second and third milligram/kgm. of histamine. The rats were allowed to survive an hour and then were sacrificed.

RESULTS. This dose level (29 mgm./kgm.) of Pyribenzamine and Antergan is not tolerated by the rat and is considerably above the LD_{50}. However, the

rats survive this dose of Benadryl and the corresponding dose of 0-2, 315 (34 mgm./kgm.). Approximately seventy to seventy-five per cent of the fall in blood pressure produced by the histamine was antagonized and about ninety per cent decreased in duration. A typical record is shown in figure 2.

Instead of investigating lower doses of these agents in rats, which are already relatively insensitive to histamine, it was considered more worthwhile to investigate the lower dose range in guinea pigs. The guinea pigs were prepared

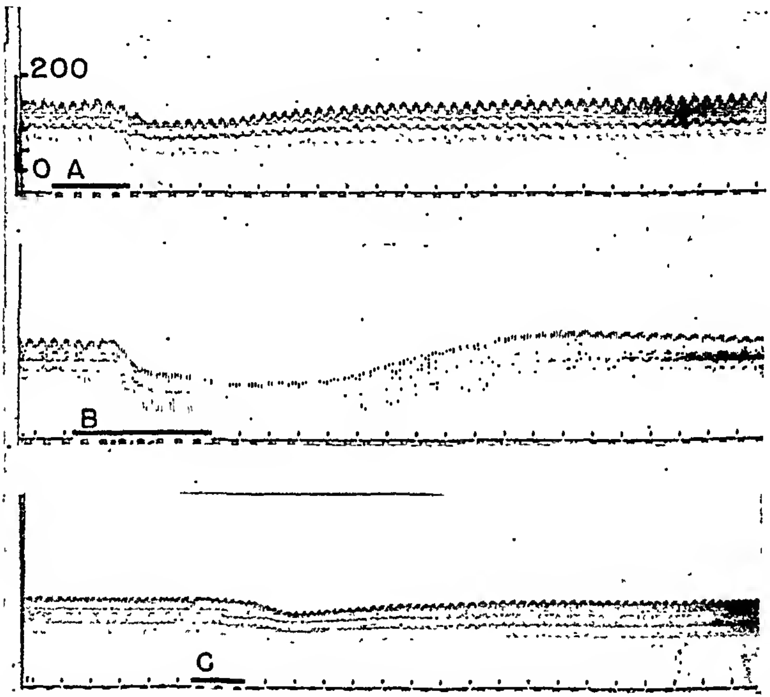

FIG. 2. 350 gram white male rat. One mgm./kgm. of histamine acid phosphate given intravenously at A and C. 34 mgm./kgm. of dimethylaminoethyl phenyl-α-thienylglycolate hydrochloride given at B. Injections given three minutes apart. Time signal 2.5 seconds.

in the same manner as the rats. Following Staub's procedure (2), no test dose of histamine was given. 5×10^{-5} Moles/kgm. of the antihistaminic agent was given intrajugularly and after three minutes, 1.7 mgm./kgm. of histamine phosphate. The guinea pigs were sacrificed after one hour.

Lehman and Knoefel (18) used a somewhat similar technic in demonstrating the antihistaminic activity of diethylaminoethyl 9,10-dihydroanthracene-9-carboxylate in which they injected the agent subcutaneously and later intracardially administered 1.7 mgm./kgm. of histamine phosphate, a dose which is

about three times the certain fatal dose. Their compound protected the guinea pigs from most of the effects of the histamine.

The results obtained from three litter-mates are given in table 3 and sample records in figure 3. Figure 3A shows that this dose of histamine can produce death in the guinea pig by direct myocardial depression, as well as by the better known bronchospasm. In contrast to this, the broadening of the pulse (systolic-diastolic difference) about twenty or thirity seconds after the administration of this dose of histamine after Benadryl or 0–2, 315 (arrow in 3C and 3D) indicates that these agents protect the heart against the effects of histamine, for the heart tends to maintain the blood pressure of the animal in the face of

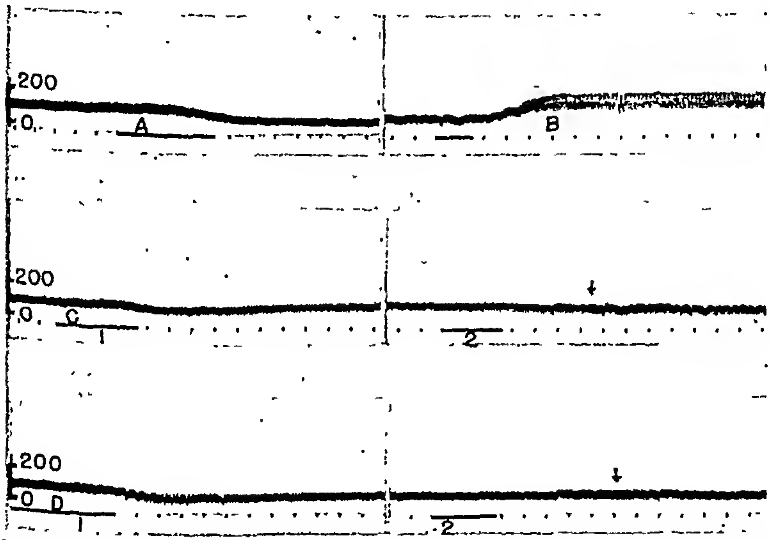

FIG. 3. Four 350 gram male guinea pigs. A received 1.7 mgm./kgm. of histamine acid phosphate intrajugularly at the time line signal; B received twenty micrograms/kgm. of epinephrine. C received 0–2, 315 17 mgm./kgm. at the first signal and three minutes later at the second signal 1.7 mgm./kgm. of histamine acid phosphate D received 14.5 mgm./kgm. of Benadryl at the first signal and three minutes later at the second signal 1.7 mgm./kgm. of histamine acid phosphate. The time line intervals are 2 5 seconds.

increased peripheral run-off and this seldom, if ever, occurs in an animal with poor cardiac function (14). It is also indicative that the doses of antihistaminic agent administered did not markedly damage the heart. No attempt was made to determine the number of lethal doses of histamine these agents would protect the guinea pigs against (4a, 5), the object being only to see the antagonism of the blood pressure effects, which are not the primary important factors in the lethal action of histamine in the cavy. Certainly considerable antagonism did occur. Double this dose of Pyribenzamine and Benadryl are immediately fatal for the guinea pig on intravenous injection.

D. Experiments with cats. Woodbury (15) demonstrated that histamine

produces pulmonary arterial vasoconstriction in the cat, and it was considered reasonable to investigate the antagonism of this phenomena with these agents. Six cats were anesthetized with 350 mgm./kgm. of sodium barbital given intraperitoneally. A cannula attached to one Hamilton manometer was introduced into the left carotid artery and a long 14 gauge sound attached to another Hamilton manometer was introduced down the right external jugular vein into the right ventricle, according to the technic described by Woodbury and

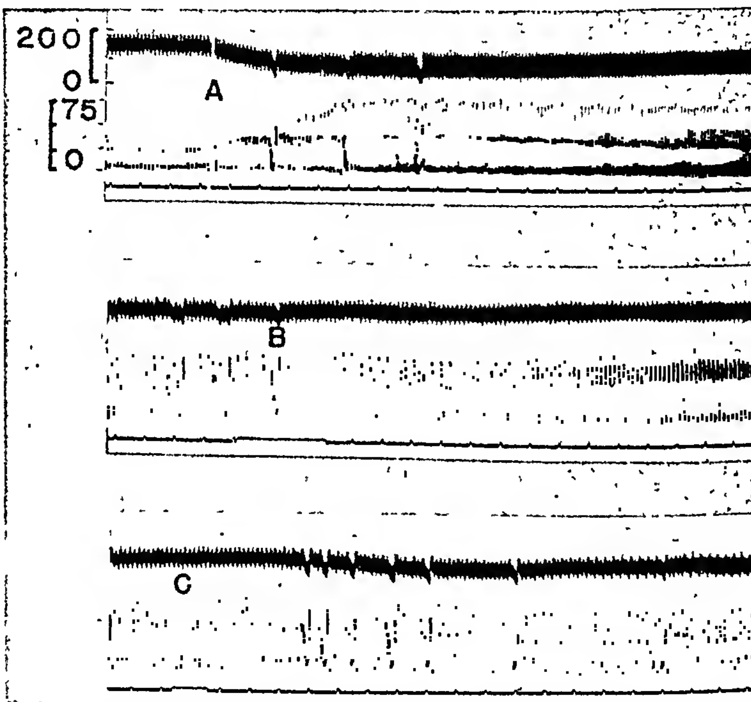

FIG. 4. Four kilogram male cat. Upper record of each of the three sections is the carotid arterial blood pressure, lower record of each of the three sections is the right heart intraventricular blood pressure. Two hundred micrograms/kgm. of histamine acid phosphate injected intravenously at A. Five minutes later 7.2 mgm./kgm. 0-2, 315 injected intravenously, and five minutes later the histamine injection was repeated at C.

Abreu (19). Two hundred micrograms/kgm. of histamine were injected, 2×10^{-5} Moles/kgm. of the agent, and the histamine repeated. In every case, the rise in the right heart intraventricular pressure produced by the pulmonary vasoconstriction was completely antagonized, but the fall in systemic arterial pressure was incompletely antagonized (see fig. 4). In attempts to increase the systemic antagonism, the dose of agent was increased to 1×10^{-4} Moles/kgm., but the systemic blood pressure was permanently depressed,

making it impossible to determine the amount of histamine antagonism produced. Larger doses of histamine were not given in cats, since they tend to produce rises in the right heart pressure by means of back pressure from a damaged left heart, and these agents could not be expected to antagonize such a secondary effect. Certainly, the antagonism produced by these agents in the cat is far inferior to that in the dog. These agents often produce a typical

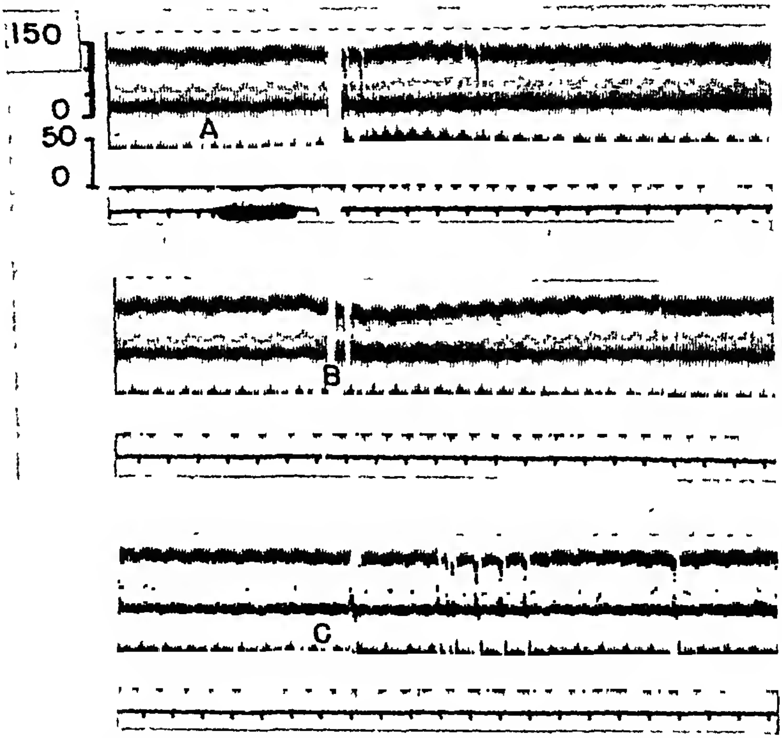

FIG 5 3 6 kilogram male rabbit Upper recording of each of the three sections is the left heart intraventricular blood pressure and the lower recording of each of the three sections is the right heart intraventricular blood pressure One hundred micrograms/kgm of histamine acid phosphate was administered intravenously at A Five minutes later, 1 45 mgm /kgm of 63C or Pyribenzamine was administered at B, and five minutes later, the histamine injection was repeated at C

transient fall and then a slight rise in systemic arterial pressure in the cat and rat, similar to that reported for similar agents in the dog (11, 16). Figure 2B is a good example of this in the rat, although it does not occur in figure 4B to any measurable degree.

It is considerably more difficult to satisfactorily introduce sounds into both the right and left ventricles of the cat or rabbit than it is in the dog. In attempts

with nineteen rabbits, ranging in weight from three to seven kilograms, only four successful preparations were made, the rest of the animals dying suddenly from inadvertant puncture of the aorta or ventricle. Since large cats are locally rare, no attempts were made to obtain left heart intraventricular pressures, simultaneously with the right heart pressures previously discussed. However, in the course of this investigation, one rabbit was adequately prepared and investigated with histamine and Pyribenzamine. Sections of this record are reproduced in figure 5. This particular rabbit exhibited both pulmonary and systemic vasoconstriction in response to histamine, and the antagonistic effects of Pyribenzamine are readily apparent even though they are incomplete.

DISCUSSION. Although all of the agents investigated possessed the ability to antagonize the histamine depressor effect for blood pressure, some of the agents were considerably more active than others. The histamine depressor effects in animals other than the dog could be at least partially antagonized, although the quantitative relationships are probably different.

The histamine depressor effect on blood pressure requires quite large doses

TABLE 3

Effect of antihistaminic agents in the guinea pig

NAME OF COMPOUND		BLOOD PRESSURE AFTER ADMINISTRATION OF AGENT			BLOOD PRESSURE AFTER ADMINISTRATION OF HISTAMINE		
		Time interval					
		Normal	10"	2'	Normal	30"	2'
0-2, 315	17 mgm /kgm.	140/90	110/50	135/85	135/85	130/60	110/50
63C	14.5 mgm./kgm.	135/85	100/0	120/70	125/75	100/50	75/25
Benadryl	14.5 mgm./kgm.	145/95	105/50	125/95	125/95	140/95	125/80

of antihistaminic agent be given to be adequately antagonized. It can be readily calculated that as little as one molecule of 63C can antagonize one molecule of histamine on isolated or intact guinea pig lung and five or six molecules of histamine on isolated guinea pig ileum (5). It is more difficult to work out the relationships from the data available for the other compounds investigated, but certainly all of them antagonize the contractile effects of histamine, be it on bronchi, or uterus, or gut, in concentrations of tens of molecules per molecule of histamine, although the relationship may be closer to a one to one differential competition for a given receptor than is apparent. In connection with this, it is interesting to note that the vascular contractile action, where it noticeably occurs in the rabbit systemic and cat pulmonary arterial system, is antagonized by lower doses of the antagonists than was expected from the data obtained on the corresponding depressor antagonism. If one calculates the relationship for the antagonism of the depressor effect of histamine, the figure approximates one thousand molecules of Benadryl to antagonize one molecule of histamine. The relationship is similar for 0-2, 315 and Pyribenzamine. As the dose of these agents is increased, considerably larger amounts of histamine

can be antagonized, and the ratio decreases to as low as one hundred to one, but still considerably out of the range for antagonizing the contractile actions of histamine on intestine or bronchi. Although the concept of competitive inhibition of receptors is the most attractive one and seems the most reasonable (7b), the differential quantitative relationship between one large group of effectors and the other group, the depressor effectors, is so wide as to be disturbing. Also, it is difficult to reconcile the incomplete antagonism these agents often produce, regardless of the dose, and the idea of only one mechanism being involved in producing the fall in blood pressure by small doses of histamine. More than one mechanism cannot be involved or the repeated administration of histamine could scarcely give the same results, nor would a symmetrical dose-effect curve. be obtained for increasing doses of histamine. However, with large doses of these agents, the deleterious effects of very large doses of histamine on the heart are also antagonized, and this may involve even a further physiological mechanism. Regardless of the mechanism of action, the agents are effective against most of the known actions of histamine.

BIOCHEMORPHOLOGY. Examination of the chemical formulas and comparison with the relative activity of the compounds might tempt one to draw certain conclusions that are not entirely justifiable. The most active member of the Pyribenzamine series is the dimethylamino compound, 63C itself, although the diethyl compound has some activity as do some other homologs. The most active member of the Benadryl series is the dimethylaminoethyl compound, Benadryl, although the diethylaminoethyl and piperidinoethyl compounds possess some activity. The same relationship holds true for the Stearns' compounds, in which the dimethylaminoethyl ester, 0–2, 315, is most active while the diethylaminoethyl and piperidinoethyl esters possess some, but less, activity. However, one must not overlook the very wide differences in the other half of the compounds: one is a derivative of 2-aminopyridine, one a derivative of diphenylmethanol, and the third an ester of phenyl-α-thienylglycolic acid. And it must be remembered that Rieveschl (17) found that very minor changes in the diphenylmethanol half of the molecule usually resulted in major loss in activity. Simple substitution of halogen, or the rearrangement of the phenyl rings to make either fluorene or naphthalene derivatives resulted in agents with little activity. With the Stearns' compounds, if the side-chain hydroxyl is removed, converting the compound from a substituted glycolic acid to a substituted acetic acid, very little activity remains. It is seldom that one finds three chemically very different compounds with similar activity in similar dose ranges. The compounds are so similar in their primary pharmacological effects that their rational clinical use will depend largely on which compound has the least undesirable side-effects.

SUMMARY

The most active known antihistaminic agents have been tested for their ability to antagonize the depressor response of histamine on blood pressure in the dog, and their activity compared on a quantitative basis.

The three most promising available compounds, Pyribenzamine, Benadryl, and dimethylaminoethylphenyl-α-thienylglycolate (0–2, 315) were shown to be able to antagonize the various cardiovascular effects of histamine in the guinea pig, rat, rabbit and cat.

ACKNOWLEDGMENTS. We are grateful to E. H. Volwiler of the Abbott Laboratories for generous samples of 929F and 1571F; to R. L. Mayer of the Ciba Pharmaceutical Company for 2339RP and 63C, and to M. L. Moore of the Frederick Stearns and Company for the rest of the agents used in this investigation, except Benadryl which was purchased on the open market.

BIBLIOGRAPHY

1. BOVET, D., AND STAUB, A. M.: Compt. rend. soc. biol., 124: 547, 1937.
2. STAUB, A. M.: Ann. Inst. Pasteur, 63: 400 and 485, 1939.
3. HALPERN, B. N.: Arch. int. Pharmacodyn., 68: 339, 1942.
4a. LOEW, E. R., KAISER, M. E., AND MOORE, V.: THIS JOURNAL, 83: 120, 1945.
4b. LOEW, E. R., MACMILLAN, R., AND KAISER, M. E.: THIS JOURNAL, 86: 229, 1946.
5. MAYER, R. L., HUTTRER, C. P., AND SCHOLZ, C. R.: Science, 102: 93, 1945.
6. LANDS, A. M., NASH, V. L., AND HOOPER, K. Z.: THIS JOURNAL, 86: 129, 1946.
7a. WELLS, J. A., AND MORRIS, H. C.: Fed. Proc., 4: 140, 1945.
7b. WELLS, J. A., MORRIS, H. C., BULL, H. B., AND DRAGSTEDT, C. A.: THIS JOURNAL, 86: 122, 1945.
8. BURN, J. H., AND DALE, H. H.: J. Physiol., 61: 185, 1926.
9. CLIMENKO, D. R., HOMBURGER, E., AND MESSER, F. H.: J. Lab. Clin. Med., 27: 289, 1941.
10. YONKMAN, F. F., CHESS, D., MATHIESON, D., AND HANSEN, N.: Fed. Proc., 4: 143, 1945; THIS JOURNAL, 87: 256, 1946.
11. SHERROD, T. R., SCHLOEMER, H. F., AND LOEW, E. R.: Fed. Proc., 5: 202, 1946.
12. RAMANAMANJARY, W.: Compt. rend. soc. biol., 138: 480, 1944.
13. SHULER, R. H., KUPPERMAN, H. S., AND HAMILTON, W. F.: Am. J. Physiol., 141: 625, 1944.
14. HAMILTON, W. F.: Am. J. Physiol., 141: 235, 1944.
15. WOODBURY, R. A., AND HAMILTON, W. F.: THIS JOURNAL, 71: 293, 1941.
16. ABREU, B. E., AND TROESCHER-ELAM, E.: THIS JOURNAL 86: 205, 1946.
17. RIEVESCHL, G.: A. A. A. S. Symposium on Histamine Antagonists. Gibson Island, Md. 1945.
18. LEHMAN, G., AND KNOEFEL, P. K.: THIS JOURNAL, 80: 335, 1944.
19. WOODBURY, R. A., AND ABREU, B. E.: Am. J. Physiol., 142: 721, 1944.

PHARMACOLOGICAL PROPERTIES OF ANTIHISTAMINE DRUGS, BENADRYL, PYRIBENZAMINE AND NEOANTERGAN

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Received for publication December 16, 1946

Comparisons have been made of the effects of antihistamine drugs on the depressor and spasmogenic actions of histamine in dogs since the resultant data would aid in evaluating such drugs relative to their use as pharmacological tools for determining the role of histamine in physiological and pathological processes. Furthermore, a study of these and other pharmacological and toxicological data with relation to therapeutic effectiveness of the various antihistamine drugs and to side-actions and toxicity should aid in selection of even more useful antihistamine drugs. The antagonism of the vascular effects of histamine assumes added significance in view of the recent reports that antihistamine drugs are therapeutically effective in allergic diseases (for references cf. 1–7) such as dermatoses, urticaria, and hay fever. Vascular reactions are undoubtedly fundamentally related to the major symptoms of allergy (8). The antihistamine drugs were also studied relative to their influence on the actions of acetylcholine and epinephrine on blood pressure, and their action on smooth muscle activity of the uterus and duodenum, since earlier studies (9–13) and our preliminary comparative studies (14) had shown that these drugs altered some actions of acetylcholine and epinephrine, both of which, like histamine, normally occur in the animal organism.

The antihistamine drugs employed[1] were β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) (12, 15–18), N-p-methoxybenzyl-N-dimethylaminoethyl α-aminopyridine$\cdot H_3PO_4$ (2786 R.P. or Neoantergan) (9–11), and a homologue of the latter, N-benzyl-N-dimethylaminoethyl α-aminopyridine hydrochloride (Pyribenzamine) (13, 19, 20). Each of these potent antihistamine agents was recently studied almost concurrently by separate groups of investigators, which fact accounts for the almost complete absence of desirable, direct comparisons of their pharmacological properties. It is regrettable that the commendable, extensive studies made with Neoantergan by Bovet and coworkers (9, 10, 11) have received so little consideration (7, 13).

Evidence now available indicates that the drugs employed possess some similar antihistamine actions. Bronchospasm induced in guinea pigs and depression of blood pressure elicited in dogs by the injection of histamine, or by histamine liberated during anaphylaxis, is readily prevented or diminished by Benadryl (12, 14–18), Neoantergan (9–11, 14) and Pyribenzamine (13, 19, 21, 22). Each drug prevents histamine from exerting its spasmogenic action

[1] Benadryl and Pyribenzamine were supplied through the courtesy of Parke, Davis and Company and Ciba Pharmaceutical Products, Inc., respectively. Neoantergan was kindly furnished by Dr. Daniel Bovet, Institut Pasteur, 26 Rue de Docteur Roux, Paris, France.

on isolated guinea pig ileum (9, 12, 13), and all exert some anti-acetylcholine action on isolated intestinal musculature. The effect of Benadryl on spontaneous or induced activity of uterine tissue has not been determined. Since Neoantergan is capable of stimulating isolated uterine tissue of the guinea pig (9), its homologue, Pyribenzamine may possess the same property even though both drugs antagonize the spasmogenic action of histamine on uterine tissue (9, 13, 14).

EXPERIMENTAL. Healthy female dogs were anesthetized with pentobarbital sodium (30 mgm./kgm., I.P.) and prepared for recording arterial blood pressure from the carotid artery by means of a mercury manometer. Respiration was recorded by a Marey tambour connected to a pneumograph fixed securely to the side of the chest. Duodenal and uterine activity were recorded by inserting a small balloon filled with water into the respective organ. The balloon was connected to a water manometer at a level of approximately 30 cm. above the organ. The contractions were relayed to a Marey tambour and recorded ona kymograph.

All drugs were administered intravenously, and unless otherwise specified, the stated doses represent amounts given per kgm. of body weight. Following the control responses to 5 to 10 micrograms of histamine diphosphate, 2.5 to 5 micrograms of acetylcholine hydrobromide and 2 to 4 doses of epinephrine hydrochloride (10 to 20 micrograms, total dose), one of the antihistamine drugs was administered in a single dose of 3 mgm. over a period of one minute. After 10 minutes the previous doses of histamine, acetylcholine and epinephrine were repeated and the responses compared with those obtained prior to treatment with the antihistamine drug.

In view of the fact that the pressor response to epinephrine was usually agumented by each of the antihistamine compounds it appeared possible that such enhanced responses might be due to vagal blocking. In order to test this hypothesis the effect of each of the antihistamine compounds on the pressor response to epinephrine (10 to 20 micrograms, total dose) was tested on three or four dogs previously atropinized (1 mgm./kgm.) and on another group of three dogs previously vagotomized. Epinephrine was usually administred three to four times before and following the antihistamine compound. The recording of duodenal and uterine activity was omitted in these experiments.

RESULTS. *A. Altered vascular responses.* Table 1 contains quantitative data relating to the effects of Benadryl, Pyribenzamine and Neoantergan on the depressor effects of histamine and acetylcholine. The average depressor response to histamine was diminished by 67 per cent under these experimental conditions following the administration of 3 mgm. of Benadryl. At this dose level the α-aminopyridine compounds were approximately as effective as Benadryl in antagonizing the depressor response to histamine.

Benadryl diminished the depressor response to acetylcholine by 41 per cent. In contrast to Benadryl, Pyribenzamine and Neoantergan did not diminish the acetylcholine-induced hypotension (table 1 and figure 1).

Following the intravenous administration of any one of the three antihistamine compounds the magnitude of the mean pressor response to epinephrine was definitely enhanced; 42 per cent for Benadryl, 45 per cent for Pyribenzamine and 28 per cent for Neoantergan (table 2, and figure 1). The pressor responses were prolonged by 50 to 75 per cent. This enhanced response to epinephrine was a consistent finding in all experiments with each of the drugs, although more variable and less in degree after treatment with Neoantergan.

Experiments were next made in four vagotomized and four atropinized dogs to determine whether epinephrine potentiation still obtained when cardiac vagal action was already eliminated in animals to be treated with antihistamine drugs. A comparison of the degree of the enhanced pressor response to epinephrine caused by antihistamine drugs in normal, non-atropinized animals with that which occurred in atropinized animals (table 2) revealed that the phenomenon still persisted and was in no instance significantly diminished in atropinized dogs. An equal number of experiments were made in vagotomized animals and the data (not included in table 2) were almost identical to, and thus in support of, that obtained in atropinized dogs. Other strong support for the belief that the potentiation of epinephrine was not referable to vagal blocking caused by antihistamine drugs is apparent in the data. Before injection of

TABLE 1

Effect of antihistamine drugs on smooth muscle activity and on depressor actions of histamine and acetylcholine

TREATMENT	HISTAMINE DIPHOSPHATE, 5 TO 10 MICROGRAMS/KGM., I.V., DEPRESSOR RESPONSES*				ACETYLCHOLINE HYDROBROMIDE, 2.5 TO 5 MICROGRAMS/KGM., I.V., DEPRESSOR RESPONSES*				DUODENUM‡	UTERUS‡	RESPIRATION
	Before	After	Difference† $M_1 - M_2 \pm$ P. E.		Before	After	Difference† $M_1 - M_2 \pm$ P. E.				
	mm. Hg	*mm. Hg*	*mm. Hg*	*per cent*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*Per cent*			
Benadryl	61	20	−41 ±5.44	−67	42	25	−17 ±5.52	−41	Decreased tone and motility	No effect	Stimulation
Pyribenzamine	59	27	−32 ±4.12	−53	41	42	+1 ±2.12	+2	Spasmogenic	Spasmogenic	Stimulation
Neoantergan	55	27	−28 ±4.82	−51	32	45	−13 ±5.08	+40	Spasmogenic	Spasmogenic	Stimulation

* Average of 8 depressor responses caused by histamine or acetylcholine before and after treatment of 4 dogs with each antihistamine drug.

† Where $M_1 - M_2 = 3 \times$ P.E., the difference was considered significant (Pearl: Medical Biometry and Statistics, 1930).

‡ Each of the antihistamine drugs usually blocked the spasmogenic action of histamine on the duodenum and uterus.

antihistamine drugs in atropinized dogs the pressor responses to epinephrine never exceeded the corresponding responses in normal dogs. Thus, if atropine itself failed to augment the pressor action of small doses of epinephrine in dogs anesthetized with pentobarbital it is apparent that the phenomenon noted after treatment with antihistamine drugs is not due to an atropine-like action.

B. Activity of the duodenum and uterus. Benadryl reduced the tone and inhibited the spontaneously active duodenum (figure 2) for 10 to 30 minutes and also prevented the spasmogenic effects of histamine on this organ. Neoantergan and Pyribenzamine also regularly antagonized the spasmogenic effects induced by the intravenous administration of histamine, but these compounds differed from Benadryl in that they induced or increased duodenal tone for 3 to 30 minutes when administered intravenously (table 1 and figure 2). This effect usually began within 2 to 3 minutes following injection. The small dose

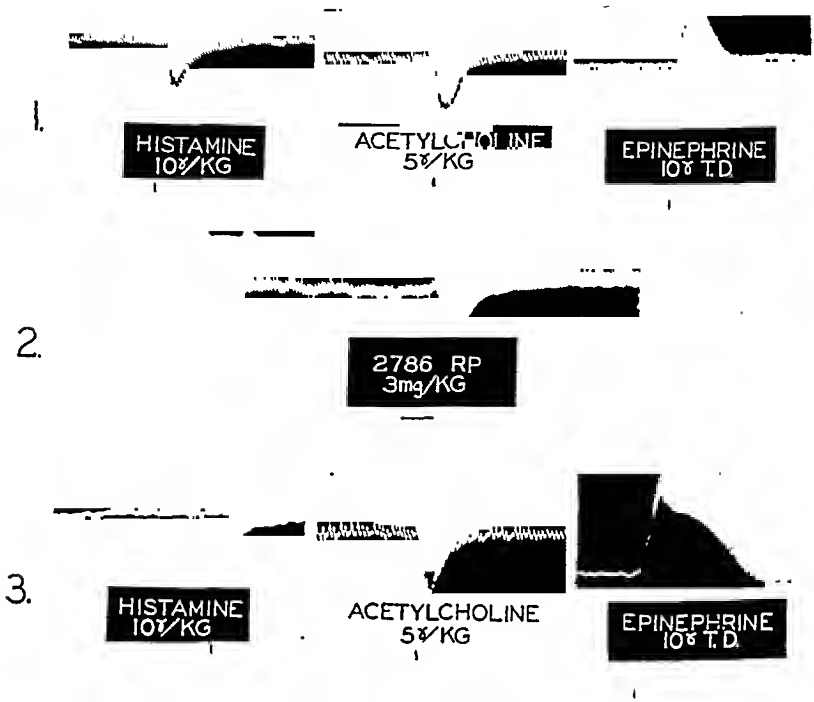

FIG. 1. BLOOD PRESSURE RECORD SHOWING THE DEPRESSOR ACTION OF HISTAMINE AND ACETYLCHOLINE AND THE PRESSOR ACTION OF EPINEPHRINE BEFORE AND AFTER 2786 R. P. (NEOANTERGAN)

Time = 5 second intervals. 1. Upper: Depressor response to histamine diphosphate and acetylcholine hydrobromide, and pressor response to epinephrine hydrochloride before 2786 R. P. 2. Center: Injection of 2786 R. P. 3. Lower: Depressor responses to histamine diphosphate and acetylcholine hydrobromide, and pressor response to epinephrine hydrochloride 10 to 30 minutes following the administration of 2786 R. P.

TABLE 2

Influence of antihistamine drugs on the pressor action of epinephrine in normal and atropinized dogs

Average pressor responses to epinephrine hydrochloride, 10 to 20 micrograms, total dose

TREATMENT 3.0 MGM./KGM., I.V.	NORMAL (4 DOGS)					ATROPINIZED‡ (4 DOGS)				
	No. of injections*	Before	After	Difference† $M_1 - M_2 \pm$ P.E.		No. of injections*	Before	After	Difference† $M_1 - M_2 \pm$ P.E.	
		mm. Hg	*mm. Hg*	*mm. Hg*	*per cent*		*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*
Benadryl	12	45	64	+19 ±4.88	+42	34	38	52	+14 ±2.48	+33
Pyribenzamine	12	40	58	+18 ±5.22	+45	18	40	67	+27 ±4.66	+68
Neoantergan	21	39	50	+11 ±1.19	+28	26	38	55	+17 ±5.48	+45

* Number of injections before and again after injection of antihistamine drugs.

† Where $M_1 - M_2 = 3 \times$ P.E., the difference was considered significant (Pearl; Medical Biometry and Statistics, 1930).

‡ Essentially identical data were obtained from an equal number of vagotomized dogs.

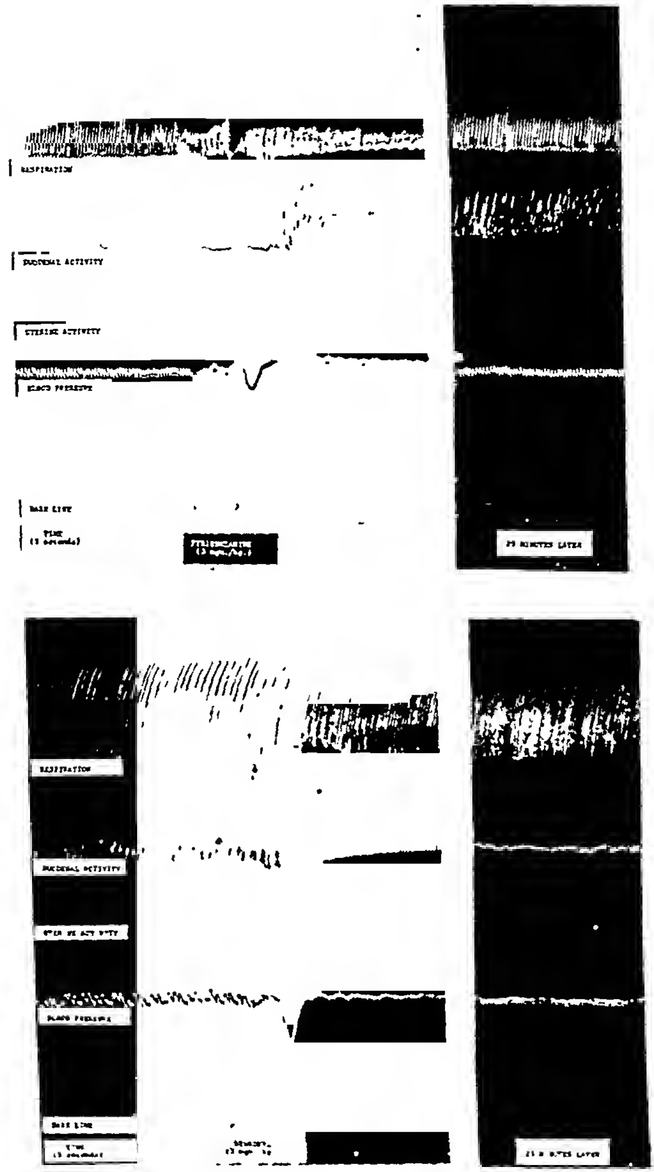

FIG. 2 1 Upper Effects of Pyribenzamine on respiration, duodenal and uterine activity, and on blood pressure. 2. Lower Effects of Benadryl on respiration, duodenal and uterine activity, and on blood pressure. (Note The small excursions in the uterine record are respiratory movements, not uterine contractions.)

of acetylcholine chosen to induce the desired depressor response only occasionally stimulated the duodenum and uterus, thus precluding the possibility of demonstrating any anti-acetylcholine action of these drugs on these organs.

With regard to the uterus, Benadryl had no regular effect on spontaneous activity, but the activity induced or augmented by histamine was blocked in three of the four experiments. In no instance did Pyribenzamine or Neoantergan reduce the spontaneous activity of the uterus, although they did oppose the spasmogenic action of histamine. The α-aminopyridine compounds differed from Benadryl in that they quite regularly induced uterine contraction when injected intravenously (figure 2). These induced contractions were equal in magnitude to those occurring spontaneously at intervals during the experiments, and persisted for approximately three minutes.

C. Bronchospasm in guinea pigs. The approximate minimal doses of Benadryl, Pyribenzamine and Neoantergan which were capable of diminishing

TABLE 3

The anti-asthmatic action and acute toxicity of antihistamine drugs

TREATMENT OF GUINEA PIGS			MORTALITY		DECREASE IN PERCENTAGE MORTALITY	P*	ACUTE TOXICITY IN MICE, $LD_{50} \pm S.E$
Drug	Mol wt	Dose	Ratio	Per cent			
		mgm / kgm , I P.					*mgm /kgm , I P*
Controls (untreated)			20/20	100			
Benadryl	291 45	1.5	16/20	80	20	0 04	75 ±5.2
Pyribenzamine	203 65	0.3	7/20	35	65	<0 001	80 ±5.8
		0.1	19/20	95	5		
Neoantergan	267 22	0 075	14/20	70	30	0 01	90 ±6.9

* P value (from Fisher's table) less than 0.05 indicates a significant difference from mortality in control group.

the incidence of mortality in guinea pigs subjected to atomized histamine were determined. Data presented in table 3, including the LD_{50} for each of the antihistamine drugs, were supplied to us through the cooperation of Dr. A. C. Bratton and Margaret E. Kaiser of Parke, Davis and Company Research Laboratories. The experimental conditions were identical to those previously employed to determine the relative potency of benzhydryl alkamine ethers (15), alkyloxytriazines (23) and other drugs (24).

On a dosage basis, Benadryl proved to be about one-fifth as potent as Pyribenzamine. These data are in general agreement with other data which indicate that Pyribenzamine is more potent than Benadryl in counteracting histamine bronchoconstriction (25), although it was demonstrated that the two drugs were equally effective in diminishing the mortality rate in anaphylactic shock. In the present experiment, the potency of Neoantergan was found to exceed that of Pyribenzamine and Benadryl four and twenty times, respectively.

There exists a close similarity in the acute toxicity of the three antihistamine drugs in mice (table 3). Five doses of each drug were injected intraperitoneally in twelve mice of both sexes, each weighing 20 grams. Deaths were recorded for five days and the LD_{50} determined from dose-mortality data graphed on log probit paper.

DISCUSSION. Hypotension induced in dogs by the intravenous administration of histamine was definitely diminished after treatment with antihistamine drugs, thus confirming earlier findings concerning Neoantergan (9–11), Benadryl (12, 17) and Pyribenzamine (26). The fixed intravenous dose of 3 mgm./kgm. for each drug resulted in essentially the same degree of antagonism of the histamine-induced hypotension. This does not permit the conclusion, however, that equal potency has been demonstrated for the three antihistamine compounds with respect to the depressor effects of histamine, since the dose of Benadryl, and possibly the α-aminopyridines, must be varied several fold in order to elicit an appreciable change in the percentage inhibition of the depressor action of histamine (12, 17). The ability of the antihistamine drugs to diminish the vascular response to histamine is of importance because of the major role of vascular reactions in anaphylaxis (27) and allergy (8).

Neoantergan and Pyribenzamine were definitely more potent than Benadryl in preventing fatal bronchospasm in guinea pigs. Variations in absorption, distribution and excretion would influence the degree of action. Relative potencies based upon a given pharmacological response may diverge widely when based upon response of a different tissue or organ. However, it is of interest to note that Pyribenzamine has recently been shown to be more effective than Benadryl in reducing the severity of histamine shock, yet no more effective in preventing fatal anaphylactic shock in guinea pigs (25) even though much evidence indicates that the severe bronchospasm occurring during anaphylaxis in this species is due to histamine (cf. 18 for refs.). In anaphylaxis, rate of histamine release and site of action may differ materially from conditions prevailing when histamine is injected or inhaled.

As previously reported (11, 12, 14, 26) each of these antihistamine compounds augment the pressor response to epinephrine. This phenomenon could be referable to a sensitization of the myocardium or arterioles to epinephrine or to the fact that the antihistamine drugs diminished or blocked vagal, cardioinhibitory impulses. The latter seemed most likely in view of the atropine-like properties of Benadryl and even possible with Pyribenzamine and Neoantergan since these compounds, like p-N-alkoxyphenylethylamines (28), could block the cardiac vagus even though failing to diminish the depressor action of acetylcholine. However, the data presented appear to rule out the possibility that the enhanced pressor response to epinephrine could be due to blocking of the cardiac vagal fibers. Yonkman *et al.* (20) sometimes noted evidence of adrenergic (and cholinergic!) potentiation relative to salivary secretion and response of the nictitating membrane in cats when injected intra-arterially with small doses of Pyribenzamine. Antergan also enhances certain responses to epinephrine (29). An obvious need exists for quantitative data and extended

of acetylcholine chosen to induce the desired depressor response only occasionally stimulated the duodenum and uterus, thus precluding the possibility of demonstrating any anti-acetylcholine action of these drugs on these organs.

With regard to the uterus, Benadryl had no regular effect on spontaneous activity, but the activity induced or augmented by histamine was blocked in three of the four experiments. In no instance did Pyribenzamine or Neoantergan reduce the spontaneous activity of the uterus, although they did oppose the spasmogenic action of histamine. The α-aminopyridine compounds differed from Benadryl in that they quite regularly induced uterine contraction when injected intravenously (figure 2). These induced contractions were equal in magnitude to those occurring spontaneously at intervals during the experiments, and persisted for approximately three minutes.

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4. Baer, R. L., and Sulzberger, M. B.: Jour. Invest. Dermat., 7: 147, 1946.
5. Decourt, P.: Bull. et mém. Soc. méd. hôp. de Paris, 59: 3, 1943.
6. Sciclounoff, R., and Junet, R.: Rev. med. de la Suisse Rom., 63: 570, 1943.
7. *Council on Pharmacy and Chemistry*, J. A. M. A., 132: 702, 1946.
8. Harkavy, J.: Arch. Int. Med., 67: 709, 1941.
9. Bovet, D., and Walthert, France,: Ann. pharm. franc., 2: suppl. to no. 4, 1, 1944.
10. Bovet, D., Horclois, R., and Walthert, France,: Compt. rend. Soc. de biol., 138: 99, 1944.
11. Bovet, D., Horclois, R., and Fournel, J.: *Ibid.*, 165, 1944.
12. Loew, E. R., MacMillan, R., and Kaiser, Margaret,: This Journal, 86: 229, 1946.
13. Mayer, R. L.: Jour. Allergy, 17: 153, 1946.
14. Sherrod, T. R., Schloemer, H. F., and Loew, E. R.: Fed. Proc., 5: 202, 1946.
15. Loew, E. R., Kaiser, Margaret E., and Moore, V.: This Journal, 83: 120, 1945.
16. Loew, E. R., and Kaiser, Margart E.: Proc. Soc. Exper. Biol. and Med., 58: 235, 1945.
17. Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, Carl A.: This Journal, 85: 122, 1945.
18. Wells, J. A., Morris, H. C., and Dragstedt, Carl A.: Proc. Soc. Exper. Biol. and Med., 61: 104, 1946.
19. Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Sci., 102: 93, 1945.
20. Yonkman, F. F., Chess, Dorothy, Mathieson, D., and Hansen, Nicoline: This Journal, 87: 256, 1946.
21. Yonkman, F. F., Hays, H. W., and Rennick, B.: Fed. Proc., 4: 144, 1945.
22. Arbesman, C. E., Koepf, G. F., and Miller, G. E.: Jour. Allergy, 17: 203, 1946.
23. Loew, E. R., Kaiser, Margaret E., and Anderson, Mona: This Journal, 86: 7, 1946.
24. Loew, E. R., Kaiser, Margaret E., and Moore, V.: This Journal, 86: 1, 1946.
25. Friedlaender, S., Feinberg, S. M., and Feinberg, A. R.: Proc. Soc. Exper. Biol. and Med., 62: 65, 1946.
26. Yonkman, F. F., Chess, Dorothy, Hays, H. W., Rennick, Barbara, and Mayer, R.: Fed. Proc., 5: 216, 1946.
27. Nolf, P., and Adant, M.: Arch. int. pharmacodyn. et therap., 72: 93, 1946.
28. Broom, W. A., and Wayne, E. J.: This Journal, 86: 83, 1946.
29. Parrot, J. L.: Compt. rend. Soc. biol., 137: 378, 1943.

studies referable to augmentation of adrenergic responses after treatment with antihistamine drugs.

Since Neoantergan and Pyribenzamine are homologous compounds one would expect a close similarity in their pharmacological properties as reported, respectively, by Bovet and co-workers (9–11) and by Mayer and his collaborators (13, 19, 20, 26). The present study revealed no qualitative differences between Neoantergan and Pyribenzamine. However, Neoantergan was more effective in preventing fatal, histamine-induced bronchospasm in guinea pigs. Compared on a weight basis the acute toxicity in mice was essentially the same for each compound.

Unlike Neoantergan and Pyribenzamine, Benadryl antagonized the depressor action of acetylcholine as demonstrated in previous (12) and present experiments. Studies on isolated guinea pig ileum (12) revealed that the atropine-like property of Benadryl was much weaker than that of Trasentin and Papaverine. Similar studies with Neoantergan and Pyribenzamine (9, 13) indicated some degree of anti-acetylcholine action. This weak and restricted atropine-like action of the α-aminopyridine compounds in conjunction with their strong antihistamine action indicates that they may be more specific histamine antagonists than Benadryl. The latter compound possesses antispasmodic properties which contrast with the tendency of Neoantergan and Pyribenzamine to act as intestinal and uterine spasmogenic agents.

The similarities and differences in properties of Benadryl, Neoantergan and Pyribenzamine should be considered when choosing antihistamine compounds for use as pharmacological tools. Following extensive clinical use, consideration of the advantages and deficiencies of the individual antihistamine compounds in relation to pharmacological properties should render it possible to develop antihistamine compounds possessing a wider range of utility and a greater degree of clinical effectiveness and safety.

SUMMARY

1. In dogs, equal doses of Benadryl, Pyribenzamine and Neoantergan were of the same effectiveness in antagonizing the depressor action of histamine, whereas the depressor action of acetylcholine was diminished only by Benadryl.

2. Pyribenzamine and Neoantergan may be more specific than Benadryl as regards antihistamine action, and are more potent relative to prevention of fatal histamine shock in guinea pigs. Benadryl exerted an antispasmodic action on the dog's intestine but not on the uterus and all the compounds diminished the spasmogenic action of histamine on the duodenum and uterus. Pyribenzamine and Neoantergan contracted the duodenum and uterus.

3. These antihistamine compounds enhanced the pressor response to epinephrine, the phenomenon being unrelated to a cardiac vagal blocking action.

REFERENCES

1. O'Leary, P. A., and Farber, E. M.: Proc. Staff Meet., Mayo Clin., 21: 295, 1946.
2. Todd, L. C.: Ann. Allergy, 4: 282. 1946.
3. Arbesman, C. E., Koepf, G. F., and Lenzner, A. R.: Jour. Allergy, 17: 275, 1946.

oxygenated (usually 5 per cent carbon dioxide in oxygen) at 45°C. and then cooled, is withdrawn from the reservoir *A* by a rotary pump (C. F. Palmer (London) Ltd. catalogue

TABLE 1

S-methyl *iso*-thiourea*	$CH_3\cdot S\cdot\ C(:NH_2^+)NH_2$
S-ethyl *iso*-thiourea	$C_2H_5\cdot S\cdot\ C(:NH_2^+)NH_2$
methylguanidine	$CH_3\cdot NH\cdot\ C(:NH_2^+)NH_2$
ethylguanidine	$C_2H_5\cdot NH\cdot\ C(:NH_2^+)NH_2$
as.-dimethylguanidine	$(CH_3)_2N\cdot\ C(:NH_2^+)NH_2$
benzylguanidine	$C_6H_5\cdot CH_2\cdot NH\cdot\ C(:NH_2^+)NH_2$
O-methyl *iso*-urea	$CH_3\cdot O\cdot\ C(:NH_2^+)NH_2$
O-ethyl *iso*-urea	$C_2H_5\cdot O\cdot\ C(:NH_2^+)NH_2$
acetamidine	$CH_3\cdot\ C(:NH_2^+)NH_2$
propionamidine	$C_2H_5\cdot\ C(:NH_2^+)NH_2$
n-butyramidine	$CH_3(CH_2)_2\cdot\ C(:NH_2^+)NH_2$

*Since the amidine derivatives referred to are 99–100 per cent ionized in aqueous solution at pH7, it is convenient and not misleading to represent them as cations. In each case it is a salt of the compound named which has been tested. For simplicity, the conventional single and double bonds have been used for the structural formula of the amidine group, although according to modern concepts, the two nitrogen atoms are probably equivalent.

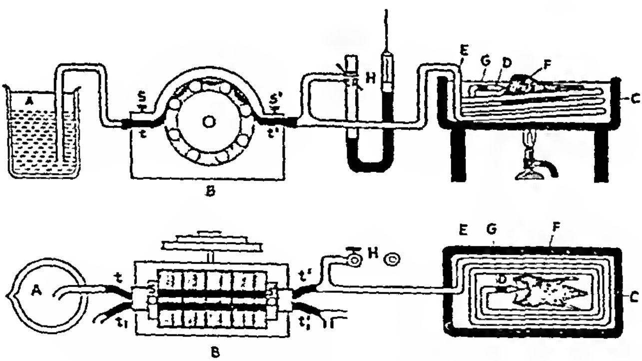

FIG. 1. CONSTANT RATE PERFUSION OF THE PITHED HIND-QUARTERS OF A RAT THROUGH A CANNULA PLACED IN THE ABDOMINAL AORTA

The upper diagram is the elevation, the lower the plan. *A* is a beaker containing the perfusion fluid, *B* a Palmer rotary pump, *H* a mercury manometer for recording changes in the perfusion pressure and *C* a heated pyrex dish containing warm Ringer-Locke solution in which is immersed a glass coil *E* carrying the perfusion fluid by the cannula *G* to the rat hind-quarters *F*.

no. F. 31), driven at a slow speed. It was found desirable to reduce further the output of the pump *B* by using rubber tubing of narrow bore. The resulting output in various experiments was 4 to 14 cc. per minute. To prevent the tubes (tt', t_1t_1') being dragged

CIRCULATORY PROPERTIES OF *iso*-THIOUREAS, GUANIDINES, *iso*-UREAS AND AMIDINES

F. N. FASTIER AND F. H. SMIRK

With successive collaboration of R. CRAWFORD AND D. U. STRANG

From the Department of Medicine, University of Otago, New Zealand

Received for publication November 14, 1945

It was shown in earlier papers that S-methyl *iso*-thiourea sulphate causes sustained rises of blood pressure especially when given intravenously (1, 2). Recently this substance has been used as a pressor agent to counteract the fall of blood pressure in spinal anaesthesia (3). Hueper and Ichniowski (4) found it more effective in dogs than ephedrine, tyramine or pituitrin when each was combined with colloidal methyl cellulose for the treatment of histamine shock.

A preliminary examination of some eighty *iso*-thiourea derivatives indicated that a number of the homologues of S-methyl *iso*-thiourea produce similar effects. Similar pharmacological activity was noticed in particular with those homologues in which some other alkyl group was attached to the sulphur atom in place of the methyl group (5). To see if possession of these properties is confined to *iso*-thiourea derivatives, chemical relatives of S-methyl *iso*-thiourea were tested in which other alterations had been made in the molecule.

This work drew attention to a resemblance between the pharmacological behaviour of similarly constituted *iso*-thiourea and guanidine derivatives. Perhaps the most obvious similarity is that several members of both groups of compounds have a well-defined pressor action which seems to depend in part if not entirely upon a direct action on blood vessels. According to published work, together with experiments reported here, analogies are provided also by their effects on intestine, uterus, the respiratory movements, the blood sugar and the sensitivity to the vasoconstrictor action of adrenaline.

The resemblance of certain *iso*-thioureas to similarly constituted guanidines led us to examine some of the corresponding *iso*-ureas and amidines. As the evidence presented will show, the close chemical relationship of the 11 substances tested (table 1) is reflected in their pharmacological behaviour. Analogies found to exist in their circulatory and other effects are so striking that it seems desirable to draw attention to these particular amidine (—C(:NH)NH_2) derivatives as a clearly defined pharmacological group.

METHODS. Dogs and cats were anaesthetized with sodium barbitone. Cannulae were placed in a femoral vein and a carotid artery, changes of pressure in the latter being recorded manometrically. Effects upon the respiratory movements were studied by connecting a stethograph, which was placed around the animal's chest, to a tambour. Isolated rabbit intestine was suspended in Ringer-Locke solution at 39°C. in an organ bath similar to that used by Burn and Dale (6).

A method for perfusing the pithed hind-quarters of the rat at constant pressure through the abdominal aorta has been described previously (5). The technique has now been modified for perfusion at constant rate (fig. 1). Ringer-Locke solution, which has been

oxygenated (usually 5 per cent carbon dioxide in oxygen) at 45°C and then cooled, is withdrawn from the reservoir *A* by a rotary pump (C. F. Palmer (London) Ltd. catalogue

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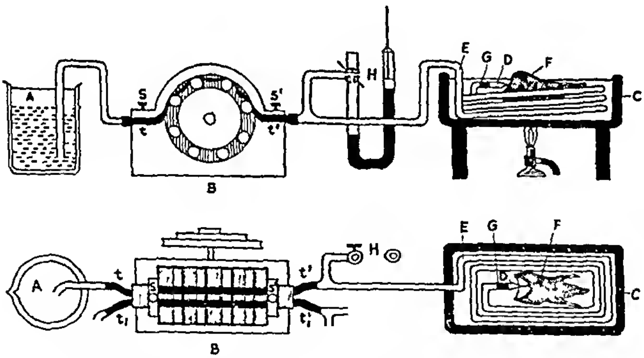

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along by the rollers, they were bound together with sticking plaster outside the adjustment screws S,S'. By using this device in place of the two Y-tubes supplied with the instrument, it was possible to perfuse simultaneously two preparations, one of which could be used as a "control". Before reaching the perfusion cannula D placed in the abdominal aorta, the Ringer-Locke solution is heated to 39°C. by passing through a glass spiral E which lies in a rectangular Pyrex dish C. This dish contains warm Ringer-Locke solution, which also serves to keep the preparation F at body temperature. It was found by experiment that the temperature and rate of perfusion could be kept practically constant. When a vasoconstrictor substance is injected into the short rubber tube G, the perfusion pressure is increased because of the greater resistance offered by the blood vessels of the rat's hind-quarters. The consequent rise in "blood" pressure is recorded with a mercury manometer H registering on a kymograph. Typical tracings are shown in figs. 3 and 4. Since the blood vessels appear, ordinarily, to be fully dilated, the preparation cannot be used for the study of vasodilator effects unless a vasoconstrictor agent is added to the perfusion fluid. It should be mentioned here that the rat hind-quarters preparation is very sensitive to pH and as aqueous solutions of most of the salts tested have an acid reaction these were dissolved in Ringer-Locke solution and neutralized with sodium bicarbonate before injection. The magnitude of the effects produced by injections of the various substances depends to some extent on the rate of perfusion, the larger effects being associated with the slower perfusion rates.

The sulphates of S-methyl *iso*-thiourea (M.P. 243°C.), methylguanidine (M.P. 238°C.), ethylguanidine (M.P. 240°C.) and benzylguanidine (M.P. 204°C.) were obtained by standard methods. Commercial samples (Kodak) of S-methyl *iso*-thiourea sulphate, guanidine acetate, methylguanidine sulphate and nitrate and *as*.-dimethylguanidine nitrate were also tested.

The amidines and *iso*-ureas used were made for us in this laboratory by Mr. P. A. Ongley, B.A., M.Sc. Acetamidine hydrochloride (M.P. 162°C.), propionamidine hydrochloride (M.P. 129°C.) and *n*-butyramidine hydrochloride (M.P. 93°C.) were prepared by standard methods. O-methyl *iso*-urea methyl hydrogen sulphate (M.P. 108°–109°C.) and O-ethyl *iso*-urea ethyl hydrogen sulphate (M.P. 69°C.) were prepared by heating together urea and the corresponding dialkyl sulphate (7), crystalline products being obtained, apparently for the first time, by this procedure. Particulars will be given elsewhere (8).

RESULTS. *Effect on the blood pressure.* As will be seen below all compounds referred to in table 1 exhibited pressor activity.

Iso-thioureas. Reference to their effects on blood pressure has been made in a previous publication.

Guanidines. Rises of blood pressure were nearly always observed in dogs and cats following the injection of guanidine and its methyl, ethyl and *as*.-dimethyl derivatives. It was found, in agreement with Alles (9), that the nature of the response to different derivatives varies considerably. The best pressor responses were obtained with methyl and *as*.-dimethylguanidine. Thus in a series of 5 vagotomized dogs, the latter (3–10 mgm./kgm.) produced rises of blood pressure which were often equal in height to those described previously with S-methyl and S-ethyl *iso*-thiourea salts; but commonly the increases were of shorter duration (fig. 2). When smaller doses of *as*.-dimethylguanidine were given, the rise of pressure was preceded by a distinct but temporary fall.

It has already been reported that certain *iso*-thiourea derivatives display the phenomenon of tachyphylaxis: that is, the pressor responses to several equal doses given in succession diminish rapidly so that a third or fourth dose of the

iso-thiourea may produce little or no rise of blood pressure or even a distinct fall. Some guanidines, but certainly not all those tested, resemble the *iso*-thioureas in this respect. Alles (9) states that large doses of ethylguanidine "may exhibit a reversal of effect and may cause a fall in pressure, especially with repeated doses or after the injection of other guanidine compounds". Similar results have now been obtained with benzylguanidine sulphate. Initial doses of this compound produced in 5 dogs biphasic pressor responses. Later doses had smaller pressor effects and a stage was soon reached when only falls of blood pressure resulted. Moreover, it was found in 4 out of the 5 experiments that, at this point, injections of methyl- and of *as*.-dimethylguanidine now failed to raise the blood pressure.

Iso-ureas. In 5 experiments on cats and in 4 experiments on dogs, the initial doses (4–10 mgm./kgm.) of O-ethyl *iso*-urea ethyl hydrogen sulphate produced rises of blood pressure which ranged from 15–50 mm. in height and commonly lasted for 10–20 minutes. Slowing of the pulse and slight stimulation of the respiratory movements were usually observed at the same time. Unlike acetamidine and propionamidine but like certain *iso*-thioureas and guanidines, this compound displayed tachyphylaxis to a considerable extent, later doses frequently having much less effect upon the blood pressure than the initial one. The sensitivity to S-methyl *iso*-thiourea also appeared to be reduced by previous injections of O-ethyl *iso*-urea. In many experiments on anaesthetized dogs and in 5 experiments on pithed cats, definite pressor responses were obtained also with O-methyl *iso*-urea.

Amidines. Rises of blood pressure which were prompt and considerable if somewhat transitory were usually observed in anaesthetized dogs and cats following the intravenous injection of either acetamidine or propionamidine hydrochloride (5–20 mgm./kgm.). Equal doses given within 5–10 minutes of each other normally gave almost equipressor responses in the same animal. The pressor effect of acetamidine was no less evident in pithed cats. Hence it may be surmised that its pressor action, like that of O-methyl *iso*-urea and S-methyl *iso*-thiourea, does not depend upon the integrity of the central nervous system. Slight pressor activity was exhibited by *n*-butyramidine in the whole animal.

Effect on blood vessels perfused with Ringer-Locke solution. When pithed rat hind-quarters are perfused at a constant rate, vasoconstriction is indicated by a rise of pressure in the perfusion fluid which would correspond to a rise of blood pressure in the whole animal. Definite increases in the perfusion pressure were produced by buffered solutions of the following salts:—S-methyl *iso*-thiourea sulphate (15 experiments), methylguanidine nitrate (12 experiments), *as*.-dimethylguanidine nitrate (9 experiments) and benzylguanidine sulphate (6 experiments). In some experiments, the salt (0.1–1.0 mgm.) was dissolved in a small quantity of Ringer-Locke solution and injected into the rubber tubing near the perfusion cannula; in others, it was dissolved in the Ringer-Locke solution contained in the reservoir and perfused in known concentration. The concentration tried was usually 1 in 10,000 but vasoconstriction was sometimes quite apparent when more dilute solutions (1 in 100,000 or less) were used.

Carefully buffered solutions of O-methyl and O-ethyl *iso*-urea (usually 0.05

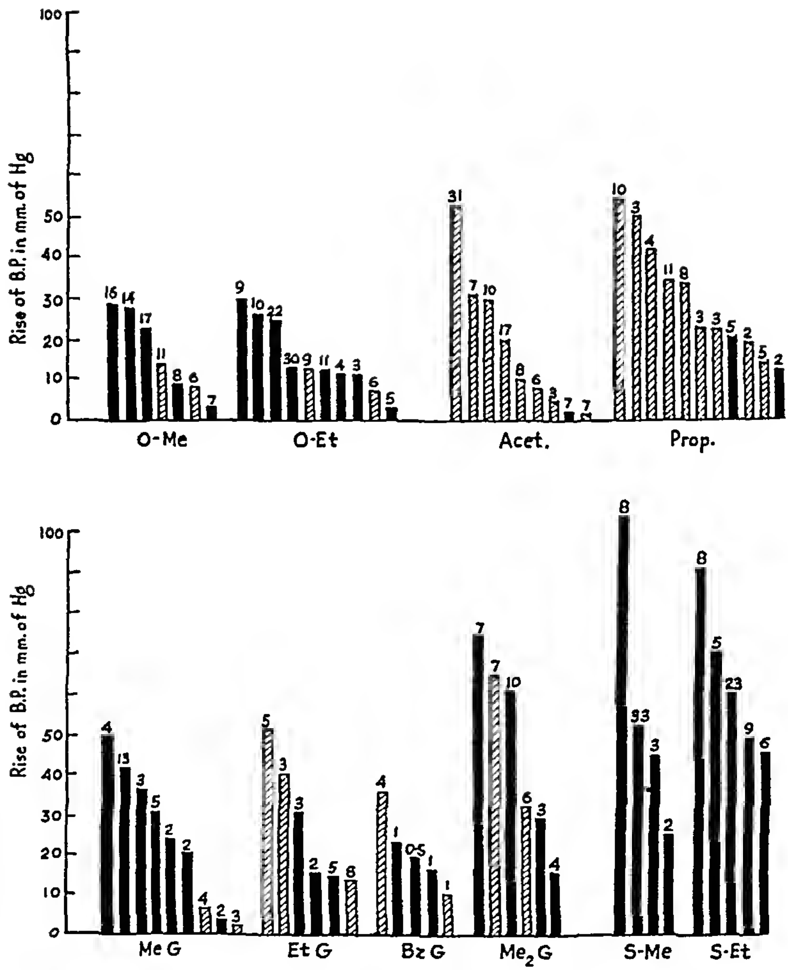

FIG. 2. Effects of O-methyl and O-ethyl *iso*-urea (O-Me, O-Et), acetamidine and propionamidine (Acet., Prop.), methyl-, ethyl-, benzyl- and *as*.-dimethyl-guanidine (MeG, EtG, BzG, Me_2G), and S-methyl and S-ethyl *iso*-thiourea (S-Me, S-Et) salts on the arterial blood pressure of anaesthetized dogs and cats. The dose (in mgm./kgm.) is given above each column. Each *solid* column represents a rise lasting for at least 10 minutes. Each *hatched* column represents a temporary rise. Falls of blood pressure (not shown) were produced in one experiment (dog) with methylguanidine and in four (cats) with benzyl, guanidine. Often with the alkyl-guanidines the pressor response was biphasic; in such cases, the second and more lasting rise is illustrated.

to 0.1 cc. of 2 per cent, injected before the perfusion cannula (fig. 1)) both cause slight vasoconstriction in the perfused pithed hind-quarters of the rat. This was

observed in 9 out of 9 experiments with O-methyl and 7 out of 7 experiments with O-ethyl *iso*-urea.

When acetamidine, propionamidine or *n*-butyramidine were injected into the pithed hind-quarters of the rat in a small volume of buffered Ringer-Locke solution (0.05–0.2 cc. of 1 per cent) or perfused through the preparation in a known concentration (usually 1 in 10,000), definite increases in the perfusion pressure were recorded in 23 out of 26 experiments with acetamidine, in 9 out of 9 experiments with propionamidine and in 7 out of 8 experiments with *n*-butyramidine.

These results support the idea that the above compounds have a vasoconstrictor action which is mainly or even entirely peripheral.

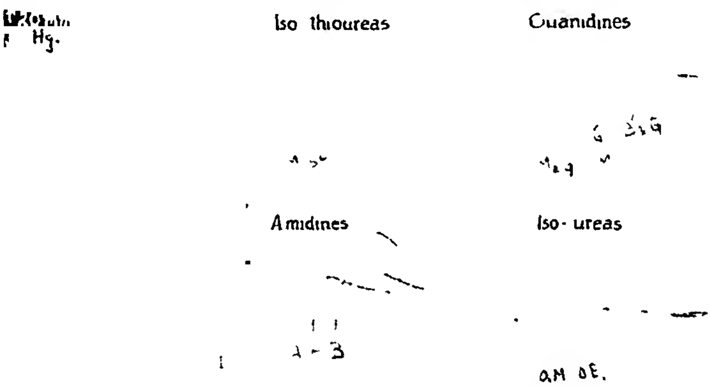

FIG. 3. PITHED RAT HIND-QUARTERS PREPARATION

On perfusion with ergotoxine (1 in 150,000) the vasoconstrictor action of adrenaline (0.4 μg. at Ad.) is soon reversed This treatment, however, does not prevent the vasoconstrictor action of the amidines and amidine derivatives tested, viz. S-methyl and S-ethyl *iso*-thiourea (S. M, S E), methyl-, ethyl-, *as.*-dimethyl- and benzylguanidine (MeG, EtG, Me_2G, BzG), O-methyl and O-ethyl *iso*-urea (O. M, O. E), and acetamidine, propionamidine and *n*-butyramidine (A,P,B) a 1 or 2 mgm dose of the neutralized salt in Ringer-Locke solution ordinarily being injected.

Effect on blood vessels perfused with Ringer-Locke solution containing ergotoxine. When the rat hind-quarter preparation was perfused with Ringer-Locke solution containing ergotoxine (0.5–3.0 mgm. in 100 cc.), the vasoconstrictor action of adrenaline was promptly reversed (fig. 3). The vasoconstrictor action of the above-mentioned *iso*-thiourea, guanidine, *iso*-urea and amidine derivatives was not abolished under these conditions (fig. 3). Thus the perfusion pressure was increased almost invariably by injections of S-methyl *iso*-thiourea sulphate, S-ethyl *iso*-thiourea, guanidine hydrochloride, methylguanidine nitrate, ethylguanidine sulphate, benzylguanidine sulphate or *as.*-dimethylguanidine nitrate, 5–7 experiments being carried out with each salt.

In 6 experiments with each salt the vasoconstrictor actions of O-methyl *iso*-urea methyl hydrogen sulphate and of O-ethyl *iso*-urea ethyl hydrogen sulphate were not abolished by ergotoxine. In 4 experiments each with acetamidine,

propionamidine and *n*-butyramidine, vasoconstriction also occurred when the compound was injected during perfusion of the rat limbs with Ringer-Locke solution containing ergotoxine.

Effect on the vasoconstrictor action of adrenaline. Experiments on dogs. The following results show that in dogs most of the amidines and amidine derivatives tested enhance the pressor activity of adrenaline in appropriate doses.

That the intravenous injection of S-methyl *iso*-thiourea, S-ethyl *iso*-thiourea and certain of their homologues make dogs and cats more sensitive to the pressor action of adrenaline (5) has been confirmed repeatedly.

Guanidine salts in doses too small to affect mammalian blood pressure have also been reported to prolong and slightly increase the pressor action of adrenaline (Burns and Watson, 10). In the present series of experiments, a definitely enhanced response to the pressor action of adrenaline was observed in dogs in 5 out of 6 experiments with *as.*-dimethylguanidine (2–7 mgm. per kgm.) and in all of 5 experiments with benzylguanidine (1–4 mgm. per kgm.). The effect of benzylguanidine in dogs differs from that in cats for only a decrease in the sensitivity to adrenaline was produced in 6 experiments on cats (0.3–10 mgm. per kgm.). Slight sensitization was noticed occasionally in experiments where the other guanidine derivatives examined were used.

In 7 out of 9 experiments O-ethyl *iso*-urea increased the sensitivity of dogs to the pressor action of adrenaline, the degree of sensitization being sometimes quite striking. O-methyl *iso*-urea methyl hydrogen sulphate likewise usually increased the pressor action of adrenaline.

In 5 out of 6 experiments on vagotomized dogs, where equal doses of adrenaline were given before and after the administration of propionamidine, the sensitivity to the pressor action of adrenaline was definitely enhanced. This effect was not observed in dogs with acetamidine but *n*-butyramidine hydrochloride increased the sensitivity of dogs to the pressor action of adrenaline to a conspicuous degree.

Experiments on perfused blood vessels. In experiments with perfused rat blood vessels, enhanced sensitivity to the vasoconstrictor action of adrenaline is exhibited at times by all and elicited regularly with most of the amidines and amidine derivatives tested (fig. 4).

In 15 out of 17 experiments on the perfused pithed hind-quarters of the rat, S-methyl *iso*-thiourea injected before the perfusion cannula (0.05 cc. of 1 per cent) enhanced the vasoconstrictor action of adrenaline. Therefore it is likely that the sensitization it produces in the whole animal depends partly or wholly upon some peripheral effect. Similar results obtained with S-ethyl *iso*-thiourea have already been reported (5).

Less definite effects were obtained with guanidines. An enhanced response in the rat hind-quarters preparation was observed with *as.*-dimethylguanidine (4 out of 5 experiments). Benzylguanidine was found to decrease the sensitivity to adrenaline in all experiments where relatively large doses (0.1 cc. of 0.5 per cent) were injected but smaller injections (0.1 cc. of 1:100,000) occasionally enhanced the response to adrenaline.

In all of 7 experiments injections of O-methyl *iso*-urea (0.05 to 0.1 cc. of 2 per

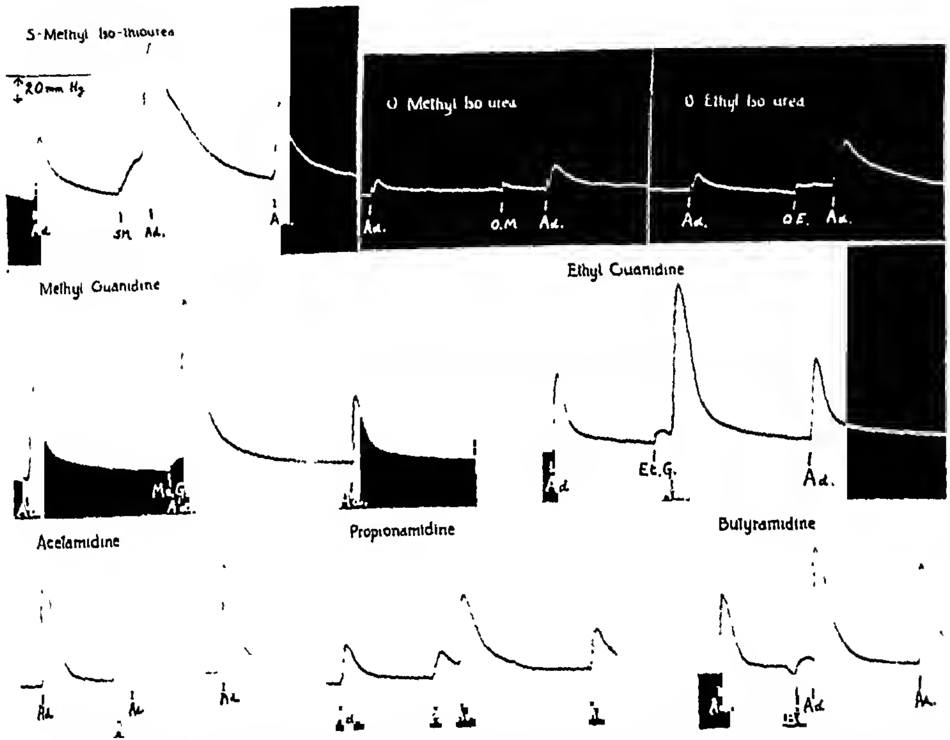

Fig. 4. Sensitization of perfused rat blood vessels to the vasoconstrictor action of adrenaline (0.4 μg. injected at Ad.) by various amidines and amidine derivatives. In the experiments illustrated salts of the following were injected: acetamidine (A), propionamidine (P), *n*-butyramidine (Bu), O-methyl *iso*-urea (O.M.), O-ethyl *iso*-urea (O.E.), methylguanidine (MeG), ethylguanidine (EtG), S-methyl *iso*-thiourea (S. M.). The doses given are as stated in the text.

cent) increased the sensitivity of the perfused rat hind-quarter to the vasoconstrictor action of adrenaline. In 6 out of 7 experiments similar amounts of O-ethyl *iso*-urea had the same effect.

Of the amidines tested, propionamidine and *n*-butyramidine when injected (0.05 to 0.1 cc. of 0.5 per cent) cause distinct sensitization. Thus 7 of the 11 substances referred to in table 1, under appropriate conditions, enhance the sensitivity of rat blood vessels to adrenaline and one is chiefly adrenalylic.

The remaining 3 substances—acetamidine (0.1 cc. of 1 per cent), methylguanidine (0.05 cc. of 1 per cent) and ethylguanidine (0.05 cc. of 0.5 per cent)—on occasion sensitize rat blood vessels to adrenaline to a definite degree. As the sensitization passes off with continued perfusion the experiment may be repeated several times on the one preparation with similar results. The individual preparation in which the sensitivity to adrenaline has been increased by one of these three substances will usually exhibit the same effect with the remaining two. In other rats, for reasons which we have been unable to determine, sensitization is

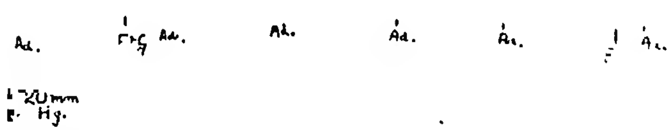

FIG. 5. Desensitization of perfused rat blood vessels to the vasoconstrictor action of adrenaline (0.4 μg. doses injected at Ad.), following the injection of ethylguanidine (0.25 mgm. at EtG).

not exhibited or an equally emphatic adrenalytic action may be seen (fig. 5) and may be demonstrated repeatedly in the same rat hind-quarters.

Effect on rabbit intestine. Although in comparison with S-methyl *iso*-thiourea sulphate, which increases the tone and strength of contraction of isolated strips of rabbit intestine in a concentration of 1:100,000 or less (2), they have a weak action on gut, guanidine and its methyl-, ethyl-, benzyl-, and *as.*-dimethyl- derivatives produce definite stimulation in concentrations of 1:50,000–1:10,000, this effect being usually reduced but not normally prevented by atropinization.

O-methyl *iso*-urea in 7 out of 8 experiments and O-ethyl *iso*-urea in 12 out of 12 experiments produced contraction of rabbit jejunum in the presence of 1:200,000 atropine sulphate. Rather larger effects were obtained in the absence of atropine. Acetamidine (15 out of 18 experiments) and propionamidine hydrochloride (all of 9 experiments) caused the contraction of isolated strips of rabbit intestine in concentrations of 1:30,000–1:10,000 in the presence of 1:200,000 atropine sulphate. Higher concentrations of atropine sulphate were used on occasion and

usually definite contractions were obtained from the various amidine derivatives.

Other pharmacological properties. Most members of the four chemical groups stimulate respiration when given intravenously in the dog. The effect is sometimes pronounced but may be transitory. The effect on the heart rate is, as one might expect, somewhat variable in the whole animal. The *iso*-thioureas and most of the guanidines studied slowed the heart rate though it was often increased

TABLE 2

Analogies in the pharmacological properties of iso-thioureas, guanidines, iso-ureas and amidines

Possession of a given property is denoted by a +, non-possession by a −

PROPERTY EXAMINED	S-METHYL *iso* THIOUREA	S-ETHYL *iso*-THIOUREA	METHYLGUANIDINE	ETHYLGUANIDINE	*as*.-DIMETHYLGUANIDINE	BENZYLGUANIDINE	O-METHYL *iso*-UREA	O ETHYL *iso*-UREA	ACETAMIDINE	PROPIONAMIDINE	*n* BUTYRAMIDINE
Rise in blood pressure in											
(a) anaesthetized animals	+	+	+	+	+	+	+	+	+	+	+
(b) pithed animal	+						+		+		
Peripheral vasoconstriction in pithed rat hind-quarters preparation	+	+	+	+	+	+	+	+	+	+	+
Vasoconstriction retained after ergotoxine in pithed rat hind-quarters preparation	+	+	+	+	+	+	+	+	+	+	+
Stimulation of smooth muscle											
(a) contraction of atropinized gut	+	+	+	+	+	+	+	+	+	+	+
(b) contraction of uterine horns	+	+	+				+				
Decreased permeability of blood vessels	+		+		+	+			+		
Tachyphylaxis shown in pressor response (dogs)	+	+	−		−	+	+	+	−	−	+
Sensitization to vasoconstrictor action of adrenaline:											
(a) enhanced pressor response in dogs	+	+	?+	?+	+	+	+	+	−	+	+
(b) enhanced response in rat hind-quarters	+	+	?+	?+	+	?+	+	+	?+	+	+
Slowing of heart rate	+	+	?+	+	?+	−	+	+	−	−	
Stimulation of respiratory movements	+	+	+	+	+	+	+	+	+	+	+

by benzylguanidine. There was usually some slowing of the heart with the *iso*-ureas and commonly an initial acceleration with the amidines.

Discussion. Analogies which have been shown to exist in the pharmacological behaviour of the 11 amidine derivatives tested are summarized in table 2. It is evident from the table that S-methyl and S-ethyl *iso*-thiourea share several of their more distinctive properties with the corresponding guanidines, *iso*-ureas and amidines.

Initial doses of the 11 amidine derivatives tested caused blood pressure increases in dogs. A peripheral site of action is indicated by the fact that all 11 substances cause vasoconstriction in perfused rat blood vessels.

Details of experiments which have been carried out with guanidine derivatives by other workers are referred to by Goldblatt and Karsner (11), who also consider that their pressor action is partly if not entirely of peripheral origin. However, finding that a large dose of ergotoxine prevents the decrease in limb volume which was observed in dogs following the intravenous injection of *as.*-dimethylguanidine, Goldblatt and Karsner concluded that the vasoconstrictor action of the latter is "brought about by stimulating the neuro-muscular apparatus of the arterioles, an action similar to that of adrenaline." Our own experiments do not bear this out. The vasoconstrictor action of *as.*-dimethylguanidine, just as with the 10 other amidine derivatives tested, was not impaired to any noteworthy extent by perfusing the rat blood vessels with a sufficient concentration of ergotoxine to reverse the action of adrenaline. The difference from our results in the rat hind-quarter may be explained by the fact that their preparation was influenced by the presence of the heart and of alternative blood depots.

Since, in addition, all the compounds were found to cause definite though occasionally slight contraction of atropinized gut, we consider that the evidence so far as it goes suggests direct stimulation of plain muscle; but whatever the interpretation of the results, it is clear that all 11 compounds produce similar effects upon blood vessels and gut when given under strictly comparable conditions.

The need for this last proviso is still more evident in experiments on adrenaline sensitization. With few exceptions the 11 amidine derivatives, in appropriate doses, sensitize dogs to the pressor action of adrenaline. Ten compounds increase, regularly in most cases, the vasoconstrictor effect of adrenaline in perfused rat blood vessels. The exact effect depends, however, upon the dose injected or concentration of the substance perfused. As will be shown in more detail in a subsequent paper (12), many *iso*-thioureas and certain other amidine derivatives enhance the response of perfused rat blood vessels to adrenaline when they are injected in small doses, produce ambiguous effects in intermediate doses and decrease the sensitivity to adrenaline in larger doses. As the range of concentrations producing sensitization varies considerably from one amidine derivative to another, sensitization may be missed if this is not borne in mind. There is also a "species" difference: thus benzylguanidine in appropriate doses increases the sensitivity of dogs to the pressor action of adrenaline whereas in cats benzylguanidine, in comparable doses, reduces it.

While 7 of the 11 substances tested, in appropriate doses, sensitize rat blood vessels regularly to the constrictor action of adrenaline, 3 of the remaining substances—acetamidine, methylguanidine and ethylguanidine—may be found either to sensitize rat blood vessels to adrenaline vasoconstriction or to reduce the sensitivity. Similar experimental conditions may produce either one effect or the other but whichever effect be exhibited it is characteristic for the particular blood vessel preparation in the sense that several repetitions of the experiment on the same vessels can be expected to yield the same results.

The last substance, benzylguanidine, strikingly reduces the sensitivity to adrenaline when injections are of the order of those used for the other substances although definite sensitization is seen sometimes when sufficiently small amounts are employed.

In short it seems that this property of sensitizing to the vasoconstrictor action of adrenaline is well developed in the *iso*-thioureas and *iso*-ureas tested and is found in some guanidines and amidines. The property has been demonstrated in the dog and in the perfused blood vessels of the rat. Certain of these substances which we refer to as sensitizing blood vessels to the vasoconstrictor action of adrenaline may, under other conditions (e.g., higher concentration and different species) reduce the sensitivity. Uncertainty of response, which is not merely a question of dosage, is encountered especially with acetamidine, methyl- and ethylguanidine. Similar experiments with guanidine and methylguanidine have been performed by Méheo and Czimmer (13), who found that these two act as synergists to adrenaline in contracting the blood vessels of frog legs. According to Burns and Watson (10), guanidine also increases the extent and duration of contraction of guinea-pig uterus treated with adrenaline.

When equal doses of S-methyl *iso*-thiourea, S-ethyl *iso*-thiourea, benzylguanidine or O-ethyl *iso*-urea are given repeatedly to the same animal the later injections produce smaller rises, or in some cases even falls, of blood pressure. This occurs, though to a less extent, with O-methyl *iso*-urea and *n*-butyramidine. That falls of blood pressure from the later injections may result from giving several doses of ethylguanidine has been reported by Alles (9).

In general all the substances tested increased respiratory movement. The effect on the heart rate is, as would be expected, somewhat variable in the whole animal. The most usual effect is a decrease in the heart rate. Similar results have been reported by Alles (9) on the guanidines which he examined.

It has been noted also (14) that acetamidine and some of the *iso*-thioureas and guanidines here studied have in common the unusual property of decreasing the permeability of blood vessels to perfusing Ringer-Locke solution.

In contrast to the derivatives here described which are of low molecular weight, various other amidine derivatives, usually of higher molecular weight, have been reported to decrease the blood pressure. The 4 aromatic diamidines with trypanocidal activity examined by Wien (15) were stated to be depressor, as were certain aryl-substituted guanidines examined by Alles (9) and by Kurodo (16) and S,S′ hexamethylene di-*iso*-thiourea (17). Of course, in so far as considerable variation in physiological activity is only to be expected in a chemical series, it is not surprising that examples can be given also of amidine derivatives (e.g. creatine, formamidine disulphide (17)) which do not display pharmacological activity of the type described. Wien (15) pointed out a resemblance of aromatic diamidines to guanidines in the antagonistic effect of calcium to their depressor action and in the sensitization of striped muscle to potassium ions. Apart from this, the only reference to the circulatory effects of *iso*-ureas or amidines was seen in a chemical paper (Easson and Pyman, 18) where it was stated that *p*-hydroxyphenyl acetamidine is pressor.

However, other effects have been investigated more fully, particularly on amidines and amidine derivatives of higher molecular weight than those we examined. Following the introduction of "synthalin" (decamethylene diguanidine) by Frank, Nothmann and Wagner (19), hypoglycaemic properties were looked for in many other guanidines and related substances. Shikinami, Yonechi, Kawai and Hosono (20), Broom (21) and others found that several types of amidine derivative have a similar kind of action, hypoglycaemia being produced especially by members with long methylene chains. When it was shown (Jancsó and Jancsó, 22) that synthalin is a trypanocide, further work (Lourie and Yorke, 23, 24; King, Lourie and Yorke, 25; Ashley *et al.*, 26) led on to the discovery of "propamidine" (4:4′ diamidino 1:3 diphenoxy propane) and similar compounds now used for the treatment of several kinds of protozoal infection. Many of the amidine derivatives examined by King, Lourie and Yorke were found to be powerful inhibitors of the Pasteur effect (Dickens, 27), while their antibacterial actions have been compared more recently by Fuller (28). Both of these workers have described analogies in the effects of corresponding guanidines, amidines and *iso*-thioureas. A possible relationship between *iso*-ureas and guanidines in their effects upon muscle tonus was suggested by Basterfield (29), who examined the central actions of a number of *iso*-urea derivatives.

The diversity of effects exhibited in various combinations by amidine derivatives of different types is noteworthy. Thus, according to their structure, they may have well defined actions on the blood pressure, the pulse, the blood sugar level and other metabolic functions, nervous irritability, muscle tonus, the sensitivity to adrenaline (and, in some cases at least, to potassium, calcium and acetylcholine) and the permeability of blood vessels. With the lower amidines and amidine derivatives of the types reported on in this paper the qualitative resemblance, pharmacologically, between corresponding *iso*-thioureas, *iso*-ureas, guanidines and amidines is close but even among higher amidines and amidine derivatives it often happens that similarly constituted compounds, whether guanidines, *iso*-thioureas or amidines, resemble each other. Superficially at least, the compounds of lower molecular weight usually differ from those of higher molecular weight. It is difficult to escape the conclusion, however, that many amidines and amidine derivatives modify the activity of many tissues and there should be a good expectation of finding related compounds with interesting and potentially useful properties.

While it cannot be stated definitely that the pharmacological activity of this group depends upon some particular chemical configuration, evidence has been collected which suggests that pressor and other characteristic properties are widely distributed amongst lower amidine (—$C(:NH)NH_2$) derivatives and are likely to be found in ones which have not yet been investigated. Moreover it would appear from the literature that a pharmacological resemblance between similarly constituted *iso*-thioureas, guanidines and amidines is not infrequent among compounds of a higher molecular weight than the substances we have

described in this paper. Further observations on this aspect of the work will be published at a later date.

SUMMARY

1. Attention is drawn to the lower members of four series of amidine derivatives—*iso*-thioureas, guanidines, *iso*-ureas and the amidines themselves—which provide close analogies in their pharmacological as well as in their chemical behaviour.

2. The present report deals especially with the circulatory properties of salts of S-methyl *iso*-thiourea, S-ethyl *iso*-thiourea, methylguanidine, ethylguanidine, *as.*-dimethylguanidine, benzylguanidine, O-methyl *iso*-urea, O-ethyl *iso*-urea, acetamidine, propionamidine and *n*-butyramidine, which differ only in the nature of the substituent attached to the amidine group.

3. All the above substances raise the blood pressure of anaesthetized dogs, produce vasoconstriction in perfused pithed rat hind-quarters even after treatment with ergotoxine, and contract atropinized gut; they appear to stimulate smooth muscle directly.

4. With few exceptions the above substances enhance the pressor action of adrenaline in dogs and, under the experimental conditions referred to in the text, all substances either increase or diminish the response of perfused rat blood vessels to injections of adrenaline. Such effects on the sensitivity to adrenaline are therefore located peripherally. In the doses employed sensitization of rat blood vessels to adrenaline is the more usual effect but one substance, benzylguanidine, reduces the sensitivity.

5. They increase pulmonary ventilation.

6. Their effect on the heart rate is not uniform in the dog but the most usual effect is slowing.

7. It is suggested that these amidine derivatives and amidines of lower molecular weight, which are closely related chemically, form a well-defined pharmacological group.

We are indebted to the Medical Research Council of New Zealand for a Grant and to the Editors of the Journal for eliminating an ambiguity.

REFERENCES

(1) Smirk, F. H.: Brit. M. J., 2: 510, 1941.
(2) McGeorge, M., Sherif, M., and Smirk, F. H.: J. Physiol., 100: 474, 1942.
(3) Smirk, F. H., and McGeorge, M.: Lancet, 2: 301, 1942.
(4) Hueper, W. C., and Ichniowski, C. T.: This Journal, 78: 282, 1943.
(5) Fastier, F. N., and Smirk, F. H.: J. Physiol., 101: 379, 1943.
(6) Burn, J. H., and Dale, H. H.: Spec. Rep. Ser. Med. Res. Counc. London, No. 69, 1922.
(7) Werner, E. A.: J. Chem. Soc., 105: 923, 1914.
(8) Ongley, P. A.: In publication.
(9) Alles, G. A.: This Journal, 28: 251, 1926.
(10) Burns, D., and Watson, A.: J. Physiol., 53: 99P, 1920.

(11) GOLDBLATT, H., AND KARSNER, H.: THIS JOURNAL, 47: 247, 1933.
(12) FASTIER, F. N., AND REID, C. S. W.: Unpublished results.
(13) MÉHEO, G., AND CZIMMER, A.: Magyar Biol. Kutatóintézet Munkái, 8: 345, 1935 (quoted by Chemical Abstracts, 30: 7226, 1936).
(14) SMIRK, F. H., AND TODD, V. E.: Unpublished results.
(15) WIEN, R.: Ann. Trop. Med., 37: 1, 1943.
(16) KURODO, A.: Folia Pharmacol. Japon., 19: 1, 1934 (quoted by Chemical Abstracts, 29: 1504, 1935).
(17) FASTIER, F. N.: Nature, London, 154: 429, 1944.
(18) EASSON, A. P. T., AND PYMAN, F. L.: J. Chem. Soc., 2991, 1931.
(19) FRANK, E., NOTHMANN, M., AND WAGNER, A.: Klin. Wchnschr., 5: 2100, 1926.
(20) SHIKINAMI, Y., YONECHI, S., KAWAI, S., AND HOSONO, T.: Tohoku J. Exp. Med., 15: 537, 1930.
(21) BROOM, W. A.: THIS JOURNAL, 57: 81, 1936.
(22) JANCSÓ, N. VON, AND JANCSÓ, H. VON: Ztschr. f. Immutitatsforsch. u. exper. Therap., 86: 1, 1935.
(23) LOURIE, E. M., AND YORKE, W.: Ann. Trop. Med., 31: 433, 1937.
(24) LOURIE, E. M., AND YORKE, W.: Ann. Trop. Med., 33: 289, 1939.
(25) KING, H, LOURIE, E. M., AND YORKE, W.: Lancet, 2: 1360, 1937.
(26) ASHLEY, J. N., BARBER, H. J., EWINS, A. J., NEWBERY, G., AND SELF, A. D. H.: J. Chem. Soc., 103, 1942.
(27) DICKENS, F.: Biochem. J., 33: 2017, 1939.
(28) FULLER, A. T.: Biochem. J., 36: 548, 1942.
(29) BASTERFIELD, S.: THIS JOURNAL, 20: 451, 1923.

THE PHARMACOLOGY OF BENZYL-IMIDAZOLINE (PRISCOL)

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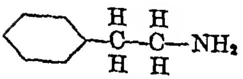
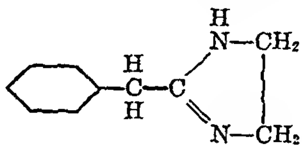
Received for publication May 22, 1946

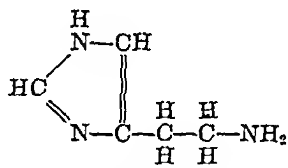
Benzyl-imidazoline hydrochloride (Priscol hydrochloride-Ciba) has been used to some extent in Europe as a vasodilator to treat various peripheral circulatory diseases (1, 2). Chemically it is related to both the sympathomimetic amines and histamine. Priscol can be regarded as a derivative of phenylethylamine in which the amine portion has been cyclized with another ethylamine group or as a derivative of histamine through the imidazole portion of the molecule. These chemical relationships are shown in the following structural formulae.



Phenylethylamine



Priscol



Histamine

The pharmacological relationships of Priscol have not been definitely established. Hartmann and Isler (3) found that Priscol lowered the blood pressure in dogs; the minimal effective dose being about 0.01 mgm. per kgm. These authors also found that it produced capillary dilation in the conjunctiva of dogs, stimulated the isolated rabbit ileum and slowed the intact frog heart. They concluded that Priscol showed many similarities to histamine. Hermann and associates (4) found that Priscol in doses of 1 to 2 mgm. per kgm. completely prevented the pressor effects of stimulation of the peripheral parts of the splanchnic nerve in dogs. Chess and Yonkman (5, 6) observed in cats that Priscol lowered the blood pressure, reversed the epinephrine pressor response, diminished or abolished epinephrine induced salivation but did not prevent epinephrine mydriasis. These effects indicate a differential adrenolytic and sympatholytic action on different organs. Bertazzi and DeGennaro (7) and Yonkman et al. (8) reported that atropine and related substances inhibited the gastro-intestinal stimulant action of Priscol. However, acetylcholine-like action upon the

cardiovascular system was found to be absent or insignificant since atropine did not prevent Priscol vasodilation in the perfused rabbit ear (9).

Since Priscol has potential therapeutic value as a vasodilator and sympatholytic agent this investigation was undertaken to study more completely its actions on the cardiovascular system and on the uterine and intestinal musculature. The experiments were done for the most part on laboratory animals, however, a few observations on humans are also included. In addition, the influence of Priscol on the actions of epinephrine and many other sympathomimetic amines has been determined.

METHODS. *Cardiovascular.* A few experiments were performed on perfused hearts from dogs, cats and rabbits. However, most of the investigations were on the intact cardiovascular system since results obtained from isolated, perfused tissues do not necessarily represent the action of the drug on the intact organism. Blood pressure was recorded by either a mercury manometer or an optical manometer (10) from the carotid or femoral artery. In some of the animals pressure pulse contours were recorded optically from the arch of the aorta by means of a metal sound introduced through the carotid artery. Blood flow was measured in various vascular beds of many of the dogs by inserting a Soskin "bubble" flowmeter (11) or a Gregg-Shipley rotameter (12) into the appropriate artery. Heparin was used as the anticoagulant in these animals. The anesthetic used varied from animal to animal. The dogs received morphine sulfate (5 to 10 mgm. per kgm.) followed by ether or sodium pentobarbital sufficient to maintain light anesthesia. The rabbits usually received only morphine but in a few experiments sodium pentobarbital was also given. Procaine infiltration was used to expose the femoral artery of the cats which were otherwise unanesthesized.

Intestine and uterus. For the isolated tissue studies, strips of ileum obtained from rabbits white rats, guinea pigs, dogs and cats, and either strips of uterus or entire uterine horns from virgin cats, rats and rabbits were suspended in a bath of Locke-Ringer's solution and the activity recorded in the usual manner. The activity of the rabbit's intestinal tract was observed fluoroscopically in unanesthetized animals given barium sulfate. In some of the dogs the activity of the ileum and nonpregnant uterus was recorded by means of a balloon and optical manometer system. These animals, being trained, were unanesthetized and the balloon was inserted into the uterus through the vagina or into the ileum through an ileostomy opening. In etherized dogs the activity of the stomach or small intestine or the pregnant uterus was recorded by a simple string and lever system through a laparotomy opening.

General. In the studies on the sympathomimetic anti-pressor action of Priscol adequate time was allowed between injections of the test amines to prevent the development of tachyphylaxis. The control responses to the long-acting amines such as ephedrine were obtained twenty four hours before the administration of the Priscol. With the shorter-acting drugs such as epinephrine or neosynephrine thirty minutes were usually allowed between recovery (return of blood pressure to the pre-injection level) and the next injection.

All of the compounds studied were used in the form of their water soluble salts, and all were injected intravenously unless otherwise stated. All doses are stated on a mgm. per kgm. basis except for the perfused heart and the human studies where they are given as total dose. For the isolated tissue studies the concentrations given represent the final concentration of the drug in the muscle bath.

RESULTS AND DISCUSSION. *Cardiovascular action.* The effect of intravenous administration of Priscol on the arterial blood pressure of dogs and cats was found to be variable. In most cats a sustained fall in pressure occurred with any effective dose (1 to 50 mgm.). In dogs, however, a marked pressor response

commonly appeared. Out of a group of twenty dogs receiving 1 to 10 mgm. of Priscol, eight showed a sustained depressor response, three showed no obvious change in mean arterial pressure, and nine exhibited a pressor response. This pressor response usually occurred with the larger doses and persisted from one minute to three hours. It was apparently not influenced by the kind of anesthesia employed, the rate of injection or the concentration of the Priscol solution used. In some animals in which the pressor phase was short in duration a sustained depressor phase followed. Since Priscol has been previously described as only a depressor substance, investigations were conducted to elucidate this above described pressor action.

This pressor action in dogs could conceivably arise from 1) sufficient vasoconstriction in some areas to more than counterbalance vasodilation in others, 2) increased blood viscosity or blood volume, and/or 3) increased cardiac output.

Vasoconstriction does not account for the pressor action as shown by direct measurements of the blood flow in various representative vascular beds and as shown by analysis of aortic pressure pulse contours. Blood flow was measured in the entire hind leg, the hind leg from which the skin had been removed, the mesentery, the kidney and the uterus (both pregnant and nonpregnant) of dogs. Priscol was administered both intra-arterially and intra-venously in doses ranging from 0.01 to 10 mgm. With the arterial administration of Priscol an increased blood flow not due to an increased arterial pressure was produced by any effective dose in all of the vascular beds studied. Some of these results are illustrated in fig. 1. On intravenous administration there was an initial decrease in flow if the arterial pressure fell. However, as soon as the Priscol had passed through the flowmeter and reached the vascular bed under investigation the blood flow then always increased. In those animals in which Priscol produced a pressor response there was an initial passive increase in blood flow due to the elevated pressure. As soon as the Priscol had passed through the flowmeter there was an additional increase in flow which was not due to the rise in arterial pressure. Indirect evidence that Priscol produced vasodilation in the skin was afforded by the fact that reddening of the skin occurred in all of the animals receiving Priscol. Evidence of vasoconstriction was not observed in any dog in any of the vascular beds studied.

Evidence of vasoconstriction could not be obtained from aortic pressure pulse contours. Analysis of these contours showed that the over-all effect of Priscol in dogs was peripheral vasodilation. At any given pressure an increase in the rate of descent of the die-away curve (diastolic slope) of the arterial pressure pulse indicates a reduction in the peripheral resistance or an increase in the "Volume Elasticity Coefficient" (13). As shown in the lower part of fig. 2, Priscol increases the rate of diastolic pressure descent at any given pressure. Therefore, Priscol must either reduce the peripheral resistance or modify the "Volume Elasticity Coefficient". The latter can be accomplished acutely only by increasing the tone of the smooth muscles of arteries, which in turn will produce a fundamental change in the type of diastolic slope (14). Since Priscol does not produce any fundamental change in the type of diastolic slope, see

fig. 2, the change in rate of pressure descent described above can be explained in only one way, namely, a reduction in peripheral resistance. This reduction in peripheral resistance produced by Priscol could arise from either peripheral vasodilation or from a reduction in blood viscosity. A reduction in blood viscosity is apparently not the cause of the decreased resistance because of the rapidity of the response and the small dose of drug employed. Certainly any reduction in blood viscosity if it did occur could not explain the pressor action of Priscol.

Changes in the standing waves of the aortic pressure pulse contour also serve

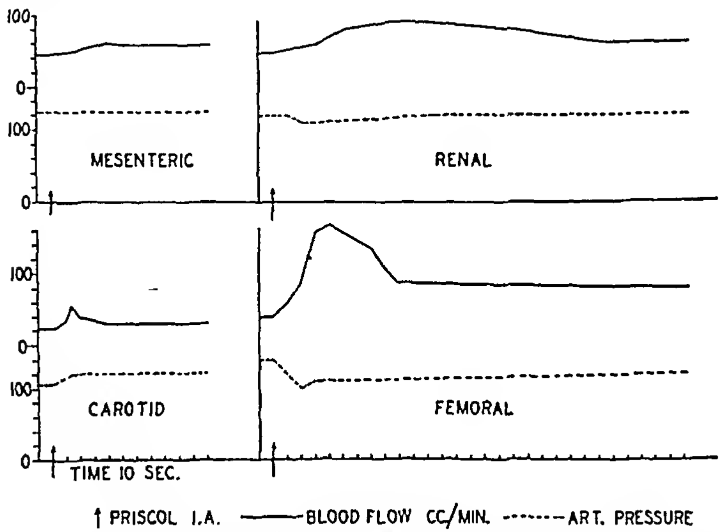


FIG. 1. THE ACTION OF PRISCOL ON BLOOD FLOW

Priscol, 1 mgm., was administered intra-arterially at each arrow. *Active vasodilation* as shown by the increased blood flow not due to increased arterial pressure occurs in all of the vascular beds. Note the variation in the degree of vasodilation; the leg and kidney showing the greatest and the mesentery and head the least. These flow studies with intra-arterial injections prove that the vasodilating action of Priscol is peripheral and not central. Vasoconstriction has not been observed in any dog with any dose used.

as critical evidence of vasodilation or vasoconstriction. It has been shown that a reduction in the amplitude and frequency of the standing waves on the diastolic slope of the aortic pressure pulse occurs with vasodilation (15). As shown in fig. 2, Priscol reduces the amplitude and frequency of the standing waves even though it raises the blood pressure. On the other hand, drugs, such as epinephrine, which raise the arterial pressure by vasoconstriction increase the amplitude and frequency of the standing waves. The changes in the aortic standing waves produced by Priscol prove that its over-all action is vasodilation.

The above data, that, irrespective of the type of blood pressure response produced by Priscol, the aortic pressure pulse contours show; 1) a reduction in

the amplitude and frequency of the standing waves, 2) a more rapid rate of diastolic pressure descent at any given pressure, and 3) no significant change in the fundamental type of diastolic slope constitute proof that Priscol reduces the total peripheral resistance in dogs by vasodilation.

Increased blood volume or viscosity are apparently not the cause of the Priscol pressor action because of the rapidity of the response and the small doses of

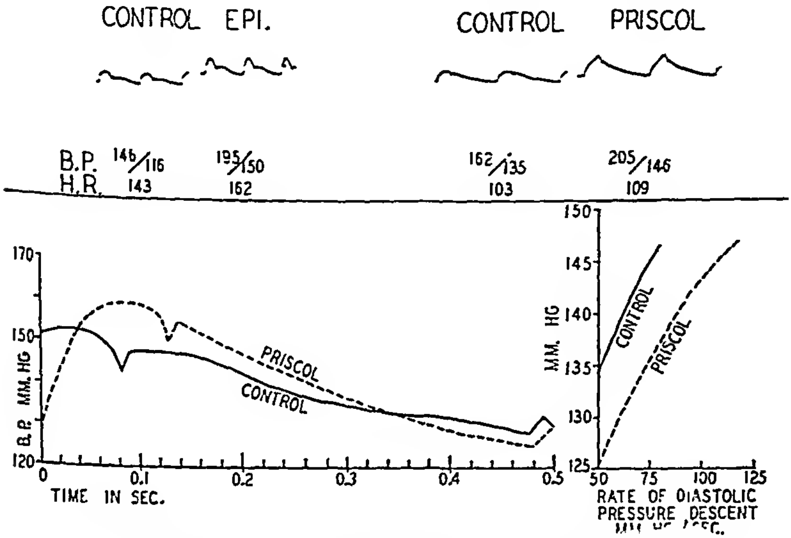


FIG. 2. ANALYSIS OF AORTIC PRESSURE PULSE CONTOURS IN THE DOG

Upper: A comparison of aortic pressure pulse contours produced by epinephrine and Priscol in the atropinized dog. The systolic and diastolic pressures and heart rate are given under each set of contours. Note the entirely different type of contours obtained during the pressor phase of these two drugs. Reflected (standing) waves which are augmented by vasoconstriction are present on the diastolic slopes of the control contours and are augmented on the diastolic slope of the epinephrine contour. They are absent, however, on the Priscol contour indicating a lengthening of the vibrating column of blood (vasodilation). These contours, being recorded from the arch of the aorta, are central in type and therefore represent changes in the *entire* peripheral vascular system.

Lower: A reconstruction of aortic pressure pulses obtained before and after Priscol. Note the absence of standing waves in the Priscol contour (see above) and the more rapid rate of diastolic pressure fall in this contour. At the right is a replot of the diastolic pressure fall of the two contours showing the rate of pressure descent at each different arterial pressure level. The control record has been corrected for the presence of the standing waves. The increase in the rate of pressure descent produced by Priscol is indicative of peripheral vasodilation.

drug employed. The volume of fluid injected was always less than 10 cc. except in two experiments in which the dose of Priscol was dissolved in 200 cc. of saline and injected slowly over a period of two hours. In one of these latter experiments the Priscol produced a slight pressor response and in the other a sustained depressor response.

Since only peripheral vasodilation has been found in dogs, even in those

showing a pressor response, the only possible explanation for the Priscol pressor action is that Priscol increases the cardiac output. Cardiac output measurements, both by the dye injection method and the analysis of aortic pressure pulse contours (16) performed by Drs. J. W. Remington and W. F. Hamilton have shown that 10 mgm. of Priscol frequently more than doubles the cardiac output.

The mechanism of the increased cardiac output was investigated by determining the effect of Priscol on the perfused heart. Coronary flow and the amplitude and rate of contraction were increased in both dog and cat hearts. One mgm. (total) of Priscol was about the optimum quantity which produced a response. (The drugs were injected into the inflow to the hearts and in these hearts 1 gamma of epinephrine produced a submaximal response when injected by this route.) Priscol also acted synergistically with epinephrine. A subminimal dose of epinephrine when perfused simultaneously with Priscol produced more marked myocardial stimulation and increased coronary flow than the Priscol alone. The response to a submaximal dose of epinephrine was prolonged and increased by Priscol. The increased cardiac output is a direct sympathomimetic effect of Priscol although increased venous return is a factor in the intact dog.

The blood pressure changes produced by Priscol in the dog and cat represent the algebraic summation of its two diverse effects, namely, 1) increased cardiac output which tends to raise the arterial pressure and 2) peripheral vasodilation which tends to lower the pressure. In most cats and some dogs the two opposing effects result in a depressor response. However, in many dogs and a few cats the resultant effect is a pressor response.

The action of Priscol on the cardiovascular system of the rabbit was found to be even more complex than in the dog or cat. The arterial pressure of the rabbit was usually elevated by Priscol though a primary bradycardia of 10 to 20 seconds duration produced a transient depressor phase. This initial cardiac slowing could be due to an acetylcholine-like action since it was completely prevented by atropine. Analysis of the aortic pressure pulses showed that the pressor action of Priscol in rabbits was due to peripheral vasoconstriction. This vasoconstriction could be histamine-like in character since the degree of pressor effect of Priscol and histamine seemed to be similar in the same rabbit. Rabbits in which histamine was very potent as a pressor agent showed a marked pressor response to Priscol. Animals in which histamine was less active also showed a lesser pressor response to Priscol. Large doses (10 to 50 mgm.) of Priscol occasionally produced a transient depressor effect even in atropinized rabbits. Cardiac slowing did not occur in these instances but the cardiac contractions were markedly weakened, see fig. 3. This cardiac depression was prevented by the prior administration of ephedrine.

The action of Priscol was investigated on seven perfused rabbit hearts. The results obtained were just the reverse of those observed in the dog or cat heart. The amplitude and rate of contraction and the coronary flow were all diminished. These effects were partially prevented by atropine and completely prevented by ephedrine or epinephrine. In the rabbit heart, as in the dog or cat heart,

Priscol acted synergistically with epinephrine. In the presence of Priscol epinephrine was more active and in the presence of traces of epinephrine Priscol produced myocardial stimulation and coronary dilation.

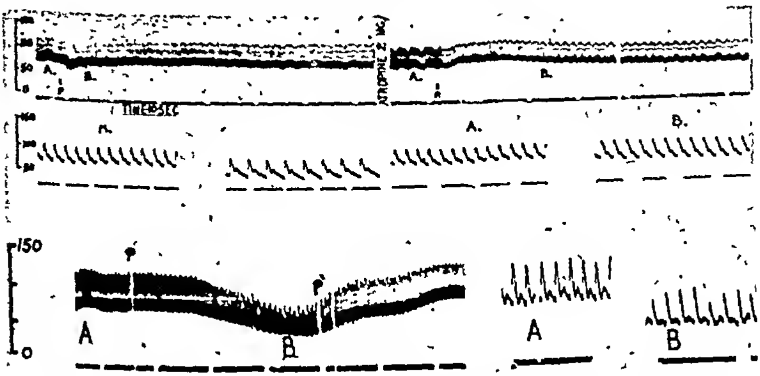


FIG. 3. THE ACTION OF PRISCOL ON THE ARTERIAL PRESSURE OF THE RABBIT

Pressure recorded optically from the femoral artery. Time intervals for the slow recordings 10 sec, for the fast recordings 1 sec Upper: The action of Priscol, 2 5 mgm administered at each mark "P", before and after atropine. Fast recordings correspond to the marked "A" and "B" sections of the top slow recording Note the transient depressor effect which is due mainly to cardiac slowing since it is prevented by the atropine Lower The action of a larger dose of Priscol in the atropinized rabbit. Fast recordings to the right correspond to the marked sections of the slow recording to the left. Priscol, 10 mgm, injected at mark "P". The reduction of arterial pressure is shown to be due to weak cardiac contractions and the reduced pulse pressure to be due to decreased cardiac output rather than cardiac acceleration

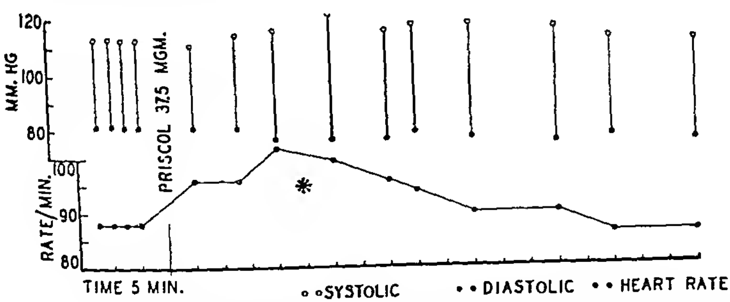


FIG 4. THE ACTION OF PRISCOL IN THE ADULT NORMAL HUMAN

Male, 90 kgm., Priscol, 37 5 mgm, administered orally Note the increase in pulse pressure associated with the increased heart rate The subjective effects noted were a feeling of fullness in the head and a sensation of body warmth. These effects were at peak intensity at the * according to the subject At this time the subject complained that it felt like "the top of his head was coming off" each time the pressure cuff on the arm was inflated.

The few humans tested showed a variable cardiovascular response to Priscol. With 25 to 50 mgm. of Priscol given orally the heart rate was unchanged or

increased, the pulse pressure always increased and the subjects usually experienced a feeling of cutaneous warmth. While both pressor and depressor responses have been observed the arterial pressure changes have never exceeded 20 mm. Hg, with the dosage employed. Fig. 4 illustrates the effect of Priscol in a male subject. These data suggest that humans may show a double response to Priscol as was observed in the dog, namely, peripheral vasodilation and increased cardiac output.

Gastro-intestinal and uterine muscular effects. The isolated ileum was both

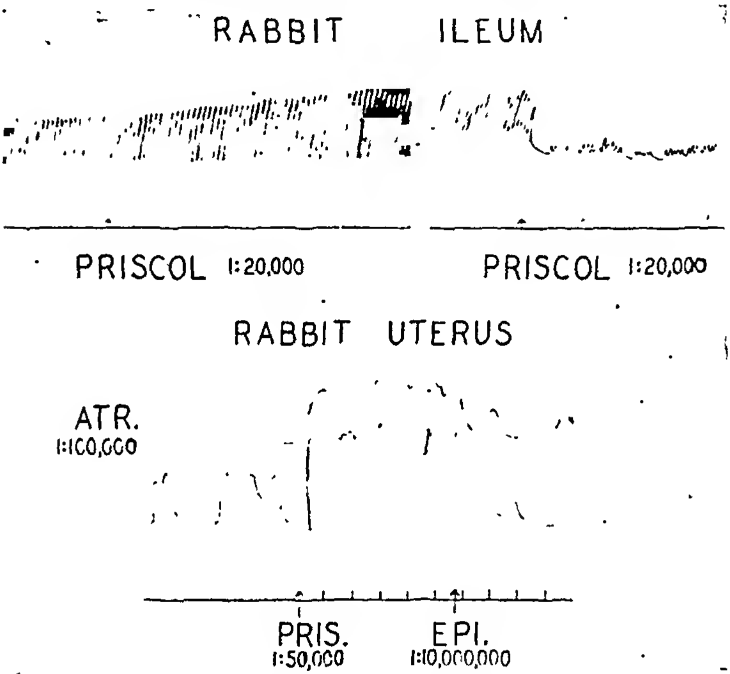


FIG. 5. THE ACTION OF PRISCOL ON THE ISOLATED RABBIT ILEUM AND UTERUS

Ileum: These tracings illustrate the extremes of action obtained with Priscol on the ileum. Uterus. The rabbit uterus was always stimulated by Priscol and atropine diminished but did not prevent this action. Time marks at 30 sec. intervals.

inhibited and stimulated by Priscol; the minimal effective concentration being about 1:100,000 in any of the various animals. The inhibitory phase appeared first being followed within 30 seconds by the stimulant phase. In some of the animals, as illustrated in fig. 5, no stimulant action was apparent. In order to characterize these actions a number of different procedures were tried. Priscol enhanced the stimulant action of barium chloride, acetylcholine and Pitressin. Atropine in concentrations up to 1:50,000 has no effect on the inhibitory action of Priscol and only slightly diminished the stimulant action. Though the action of Priscol may be partially acetylcholine-like on smooth muscles of the

intestines, it could be mainly histaminic in nature since in the histamine sensitive ileum (guinea pig) atropine inhibited Priscol and histamine stimulation to a similar extent. Priscol also augmented the action of histamine on the isolated ileum when applied either before or after the histamine. When Priscol was applied after histamine the characteristic initial depressant phase also appeared but this was followed by an augmentation of the histamine spasm.

The action of Priscol (1 to 10 mgm.) on the intact gut was also diphasic, however here the primary action was stimulation which was followed by inhibition. During the inhibitory phase further administration of Priscol did not produce stimulation. The stimulation appeared to be more intense following intravenous administration than after subcutaneous injection. In contrast to the results on isolated ileum and in agreement with the findings of others (7, 8) we found that this stimulant action of Priscol was prevented by atropine. In the intact animal therefore, Priscol apparently has some acetylcholine-like action on the gastro-intestinal tract. Apparently this stimulation is not central or ganglionic since nicotine in amounts sufficient to prevent the cardio-depressor action of the vagus did not prevent the Priscol stimulation of the gut. This stimulant action of Priscol also resembles in some respects the action of ergotamine as described by Salant and Parkins (17). These authors found that ergotamine stimulated the intact ileum and that this stimulation occurred only with the initial dose and that it was prevented by the prior administration of atropine.

The isolated uterus of the virgin cat and rabbit was always stimulated and never inhibited by Priscol, the minimal effective concentration being about 1:200,000 in the rabbit and 1:20,000 in the cat. This stimulation was only partially prevented by atropine, see fig. 5. As in the isolated ileum the stimulant action of acetylcholine, histamine and Pitressin on the uterus was augmented by Priscol. The uterus of the white rat which was inhibited by histamine was not influenced by Priscol even in concentrations as high as 1:2,000. Both histamine and Priscol however, increased the stimulant action of Pitressin in this uterus. The intact uterus of the dog and rabbit, either pregnant or non-pregnant, was mildly stimulated by Priscol. Atropine in doses up to 1 mgm. had no effect on this stimulation. As with the isolated uterus no depressant action of Priscol was seen with the intact uterus. In pregnant humans (eight months) Priscol in doses of 25 mgm. by mouth caused only slight uterine stimulation in one of two patients tested. In two dysmenorrheal patients Priscol in doses of 25 mgm. aggravated the uterine and gastro-intestinal distress.

Adrenolytic action. Priscol in suitable dosages completely blocked the pressor action of epinephrine. In dogs 10 mgm. blocked the pressor effect of 0.01 mgm. of epinephrine. It was more active in this respect in cats (about 10 times) and less active (about $\frac{1}{4}$) in rabbits. Epinephrine "reversal" occurred in those dogs which normally showed a secondary depressor response to the control dose of epinephrine, see fig. 6. Fig. 10 illustrates the type of dog in which epinephrine "reversal" did not usually occur. No "reversal" was seen in rabbits but it invariably occurred in cats.

Priscol did not show any clear evidence of an adrenolytic action on the isolated

heart. As described earlier Priscol and epinephrine were synergists on this structure. However, in one cat heart in which the control dose of epinephrine diminished the coronary flow this same dose with or following Priscol produced very active coronary dilation.

Priscol in effective concentration (1:50,000 to 1:2,000) selectively blocked the excitatory action of epinephrine on the isolated rabbit uterus. As shown in fig. 7 Priscol applied during an epinephrine induced contraction produced relaxation and epinephrine applied after Priscol did not contract the uterus until after several washings. When epinephrine was applied during a Priscol induced contraction, relaxation took place, see fig. 4. Lower concentrations of Priscol

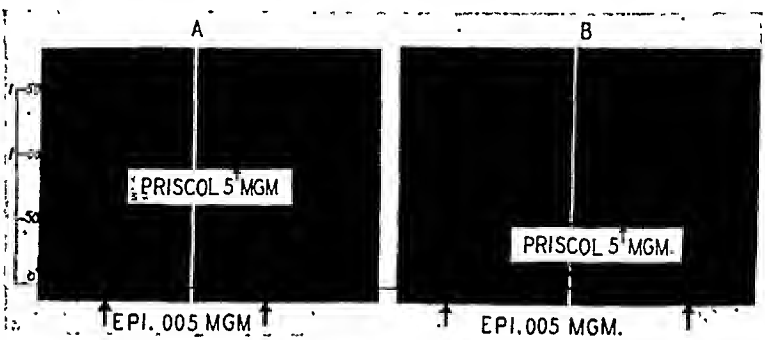


FIG. 6. THE ADRENOLYTIC ACTION OF PRISCOL

A: Dog, female, 7 kgm., morphine and ether anesthesia. Thirty minutes of the tracing have been deleted at the break. Arrows at the bottom signal injections of epinephrine. One minute after the Priscol injection the second dose of epinephrine was given. Note that the Priscol produced a transient pressor action and that epinephrine after Priscol produced a depressor response similar to the secondary fall produced by the control dose of epinephrine. B: Dog, female, pregnant, 11 kgm., morphine and ether anesthesia. Thirty minutes of the record have been deleted at the break. Two minutes after the Priscol the second dose of epinephrine was given. Note that in this animal Priscol produced only a depressor response.

merely diminished the epinephrine stimulant action. Epinephrine relaxation of the virgin cat's uterus was not prevented by any concentration of Priscol.

The intact uterus of the pregnant dog, which is normally contracted by epinephrine (0.005 mgm.), gave responses similar to those of the isolated rabbit uterus. Epinephrine administered following 10 mgm. of Priscol produced relaxation if the uterus was contracted and had no effect on the uterus if this was in a state of relaxation. When Priscol was given during an epinephrine induced spasm, relaxation occurred. Priscol, therefore, effectively blocks the excitatory action of epinephrine on the uterus thereby allowing the inhibitory action of epinephrine to appear. These results further confirm the fact that epinephrine has a dual action on all uteri (18). It may contract or relax this organ depending upon various factors including the epinephrine concentration at the site of action, the state of the uterus (pregnant or non-pregnant) and the

animal studied. Yet, irrespective of its observable effect, both actions are present though one or the other may be masked.

Priscol did not block the inhibitory action of epinephrine on the isolated ileum of the dog, cat or rat. The epinephrine inhibition of the isolated guinea pig ileum was somewhat prevented by Priscol but to no greater extent than by histamine. Only on the isolated ileum of the rabbit was any evidence obtained that Priscol could block epinephrine inhibition. In some animals and on some segments of ileum Priscol diminished the epinephrine inhibition, see fig. 8. Since this effect was so variable and because a similar effect could be produced by ephedrine or neosynephrine, we believe that Priscol even in the ileum of the rabbit does not selectively block the epinephrine inhibitory action.

Sympathomimetic anti-pressor action.[1] This part of the investigation was carried out entirely on dogs. Priscol in suitable dosage completely blocked the

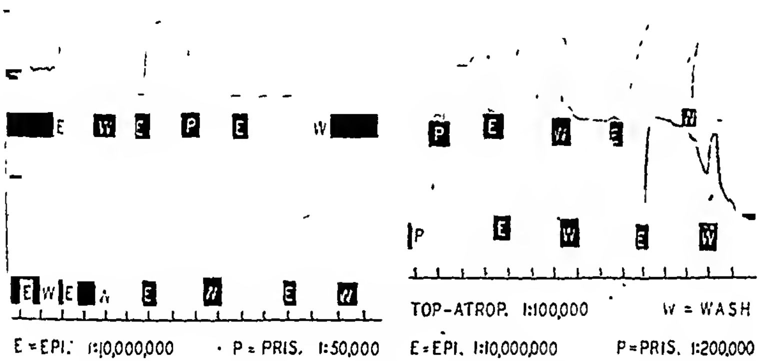


FIG. 7. THE ACTION OF PRISCOL AND EPINEPHRINE ON THE ISOLATED RABBIT UTERUS
Time marks at 30 sec. intervals. Left: The lower tracing is a direct continuation of the upper. Priscol (P) applied during an epinephrine (E) induced contraction relaxes the uterus. Epinephrine after Priscol does not contract the uterus until after several washings (W). Right: Priscol in lower concentration contracts the uterus but only diminishes the epinephrine stimulation. Atropine did not prevent any of these effects.

pressor action of all of the sympathomimetic amines available for study. Table 1 shows the relationship of the blocking action of Priscol to the pressor activity of the compounds studied. It appears that a given dose of Priscol will block a certain degree of pressor action and therefore the dose of any amine that can be blocked by Priscol is in direct ratio to the amine's epinephrine ratio.

After Priscol the action of the pressor amines varied with the compound used, see fig. 9. The amines which have little or no depressor activity (ephedrine, neosynephrine, sympatol, privine, amphetamine, propadrine, paredrine and vonedrine) had no effect on the blood pressure after a blocking dose of Priscol. Those pressor amines which have some depressor activity (epinephrine, tyramine

[1] A portion of this section has been presented to the American Society for Pharmacology and Experimental Therapeutics, Atlantic City, 1946. See Fed. Proc., 5: 161, 1946.

and 2-methylamineheptane) lost their pressor action and showed only their depressor effects.

The sympathomimetic amines which are primarily depressor compounds act in a similar manner. Ethyl norsuprarenin (Butanephrine), a depressor amine which occasionally gives a marked pressor action (see fig. 9) and which develops a pressor action on repeated administration (22) lost its pressor action after

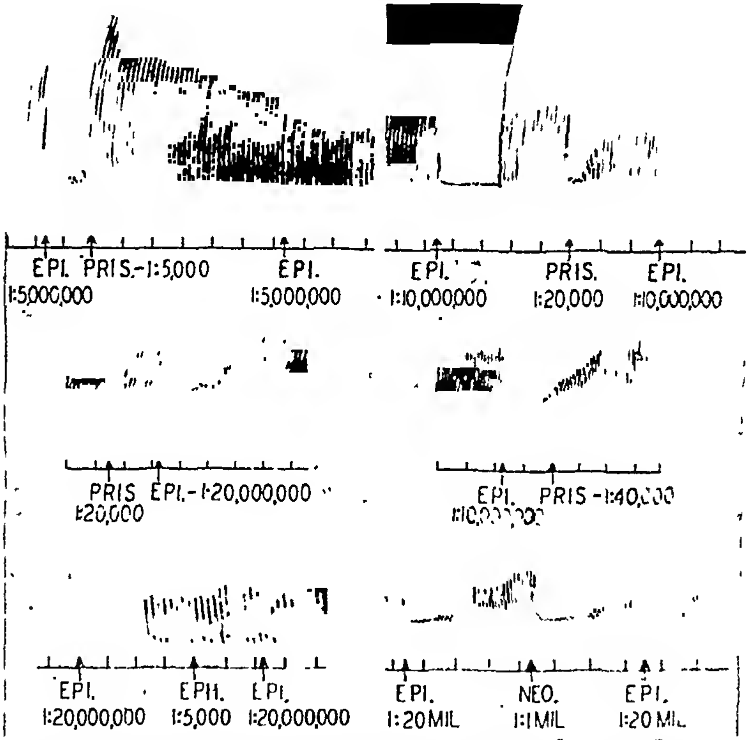


FIG. 8 THE ACTION OF PRISCOL AND EPINEPHRINE ON THE ISOLATED RABBIT ILEUM

Time marks at 30 sec. intervals. Priscol may prevent or just reduce the epinephrine inhibition of the ileum, see upper and center-left records. Priscol applied after epinephrine has only a stimulant action, see upper-left and center-right records. Ephedrine and neosynephrine although having different actions on the ileum can prevent epinephrine inhibition, see lower records.

Priscol but retained its characteristic depressor effect *even on repeated injections.* Two other depressor amines, 1-(p-hydroxyphenyl)-2-isopropylaminoethanol (#277) and 1-(3-4-dihydroxyphenyl)-2-isopropylaminoethanol (#1024), lost their slight preliminary pressor action and retained unchanged their characteristic depressor action.

Priscol did not block the pressor response to Pitressin, renin or angiotonin

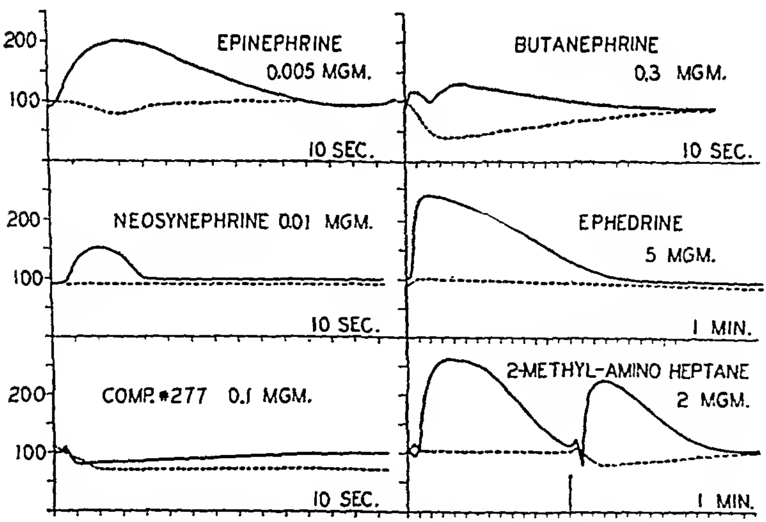
TABLE 1

A comparison between the epinephrine pressor ratio and the Priscol antipressor ratio of some of the sympathomimetic amines

AMINE	EPINEPHRINE RATIO		PRISCOL RATIO*
Epinephrine hydrochloride	1.000	(19)†	1000
Privine hydrochloride	0.2	(20)	400
Neosynephrine hydrochloride	0.02	(19)	200
Sympatol hydrochloride	0.01 -0.04	(19)	20
Tyramine hydrochloride	0.01 -0.05	(19)	10
Ephedrine sulfate	0.005-0.01	(19)	2
Propadrine hydrochloride	0.003-0.01	(19)	2
Amphetamine sulfate	0.002-0.01	(19)	2
Paredrine hydrobromide	0.01	(19)	2
Vonedrine hydrochloride	0.01	(21)	2
2-methylamino heptane HCl (EA-1)	0.01		2

* The Priscol Ratio is the approximate ratio between the dose of Priscol and the dose of amine that it will block. For example, 10 mgm. of Priscol will completely block the pressor response to 0.01 mgm. of epinephrine.

† Reference Number.



Blood pressure responses in dogs before —— and after - - - - 10 mgm. of Priscol

FIG. 9. SYMPATHOMIMETIC ANTI-PRESSOR ACTION OF PRISCOL

Arterial pressure in mm. of Hg and time as indicated on each record. These are replots from optical records showing the action of six amines on the mean arterial pressure before and after Priscol. Two injections of 2-methylamino-heptane are illustrated because the depressor effect of this amine does not become apparent unless repeated injections are made. Note the absence of pressor responses to these drugs after Priscol.

in dogs, or the histamine pressor response in rabbits. This indicates that the anti-pressor action of Priscol is selective for the sympathomimetic amines.

The relationship between Priscol and ephedrine is of great interest. Priscol usually lowered the blood pressure in cats and dogs in the presence of ephedrine. In some cases Priscol appeared to be a better depressor substance when ephedrine was present. This could be explained on a basis of "crossed tachyphylaxis" in that the presence of ephedrine diminished the cardiac stimulating (pressor)

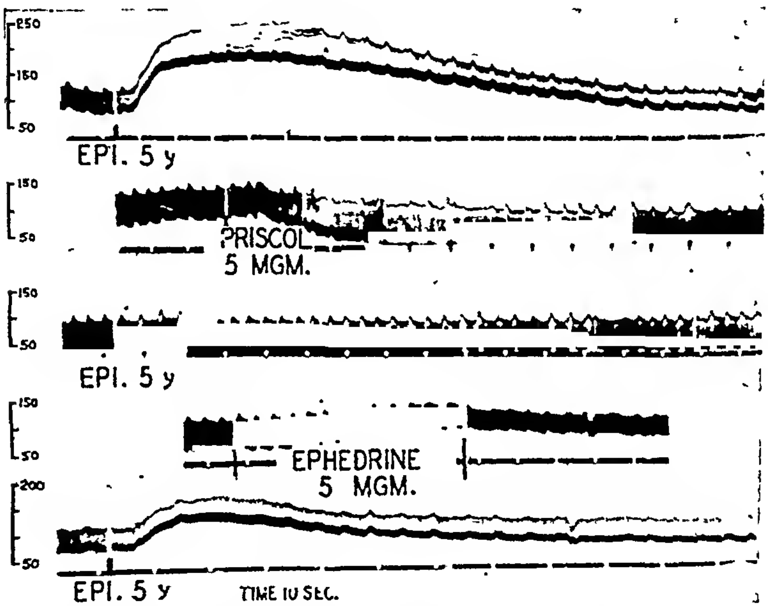


FIG 10 ANTI-ADRENOLYTIC ACTION OF EPHEDRINE

Blood pressure recorded from the femoral artery of a dog Time marks at 10 sec intervals The three epinephrine injections (see 1st, 3rd, and 5th records), were given 30 minutes before and 2 and 17 minutes after the Priscol The ephedrine was administered 5 minutes before the last epinephrine injection Note that ephedrine caused the pressor response of epinephrine to reappear In a control experiment in the same dog four days previous it was found that the epinephrine pressor response did not start to reappear until at least 60 minutes after this same dose of Priscol

action of Priscol. Likewise the Priscol diminished the cardiac stimulating action of ephedrine which would account in part for the ephedrine anti-pressor action of Priscol.

Ephedrine also caused the pressor action of epinephrine to reappear after this had been blocked by Priscol. A dose of ephedrine equal mgm. for mgm. with the dose of Priscol will restore 30 to 50 per cent of the epinephrine pressor action, see fig. 10. This action of ephedrine appeared to be more or less selective for epinephrine and was not shared to any great extent by any of the other amines

tested. Three possible mechanisms could be offered to explain this action: 1) resensitization of the pressor receptors by ephedrine, 2) blocking of the epinephrine depressor mechanism, or 3) protection of the epinephrine from enzymatic destruction thereby increasing and prolonging its action. After Priscol had brought about epinephrine "reversal" a pressor effect could be elicited by increasing the dose of epinephrine ten to one hundredfold but this was followed by a very marked depressor phase. When the epinephrine pressor response was restored by ephedrine the depressor phase was absent. These observations are against ephedrine merely protecting the epinephrine.

SUMMARY

The pharmacodynamic relationships of Priscol are very complex. In some of its actions Priscol resembles the sympathomimetic agents, in others it resembles histamine or acetylcholine, and in still others Priscol resembles the sympatholytic substances such as ergotamine or ergotoxine. Each of Priscol's diverse effects will be considered individually.

1. Peripheral vasodilation. Priscol produces general vasodilation and increased venous return in dogs and cats (and probably humans). This dilation is not prevented by atropine or by sympathomimetic agents and therefore is probably not due to acetylcholine-like or histamine-like action. This peripheral vasodilating effect of Priscol appears to be a sympathomimetic effect in a manner similar to other sympathomimetic substances including 3-4-dioxyephedrine and ethyl epinephrine (23), ethyl-norsuprarenin (22) and 1-(p-hydroxyphenyl)-2-isopropylamino ethanol (24). (Another indication of the essential sympathomimetic nature of Priscol is the fact that certain chemical modifications of the Priscol type structure produce very active sympathomimetic pressor agents such as Privine (3, 20, 25).)

2. Cardiac stimulation. Priscol stimulates the intact or isolated heart of dogs and cats. We have shown that this cardiac stimulation is capable of producing a pressor response in many dogs by increasing the cardiac output; the increase in cardiac output being sufficient to increase the arterial pressure even in the face of general peripheral vasodilation. This cardiac effect of Priscol is probably sympathomimetic in nature and appears to be due both to a direct myocardial effect and to active coronary dilation.

3. Pressor action in rabbits. The arterial pressure of rabbits is usually increased by Priscol. This action is apparently histaminic in nature since there is a correlation between the intensity of the histamine pressor action and the Priscol pressor action in the same animal. Pulse contours show that Priscol (and histamine) increases the total peripheral resistance in rabbits. In dogs and cats the total peripheral resistance is diminished.

4. Cardiac depression in rabbits. On the isolated or intact rabbit heart, Priscol produces two different types of transient depression. One is acetylcholine-like in nature since it is prevented by atropine. The other occurs only with large doses and is apparently histaminic since it occurs in the presence of atropine but is prevented by epinephrine or ephedrine.

5. Gastro-intestinal action. The principle action of Priscol on the gastro-intestinal musculature is stimulation. In the intact animal the stimulation is prevented by atropine but on the isolated gut atropine does not prevent the stimulation. The stimulant action of Priscol on the isolated gut appears to be histaminic in nature since the histamine sensitive ileum (guinea pig) is also more sensitive to Priscol. Priscol also produces some inhibition of the gastro-intestinal musculature. This inhibition precedes the stimulation phase in the isolated gut and follows the stimulant phase in the intact animal. With some segments of isolated ileum only inhibition is noted. During the inhibitory phase in the intact animal, especially following large doses of Priscol, the gut appears to be resistant to further Priscol stimulation. This inhibitory action of Priscol could be sympathomimetic in nature.

6. Stimulation of the uterus. Priscol stimulates the intact uterus (pregnant or non-pregnant) of the dog and the isolated uteri of cats, rabbits and guinea pigs. Atropine does not prevent this stimulation. Although this action of Priscol resembles that of histamine in the intact animal the stimulation is much less marked.

7. Sympatholytic action. Priscol effectively blocks the pressor action of epinephrine in experimental animals. This effect is produced by blocking the peripheral vasoconstricting action of epinephrine since it has been shown that epinephrine following Priscol is an active vasodilator (26) and that Priscol does not prevent the cardiac stimulation produced by epinephrine. Priscol also blocks the excitant action of epinephrine on the uterus. This allows the uterine inhibitory action of epinephrine to become apparent.

The sympatholytic action of Priscol is similar to that of the ergot alkaloids, ergotoxine and ergotamine. However, in its cardiovascular effects Priscol is entirely dissimilar in action. The ergot alkaloids produce a pressor effect by vasoconstriction. This usually results in a diminished cardiac output. Priscol, on the other hand, increases cardiac output and thereby produces a pressor effect in many dogs.

8. Sympathomimetic anti-pressor action. Priscol selectively blocks the pressor effects of the sympathomimetic amines. It has no effect on the pressor actions of Pitressin, angiotonin, renin or histamine. The amount of amine which is blocked by Priscol varies with the pressor potency of the amine. Only those amines which possess some masked depressor action (epinephrine and tyramine) show "reversal" after Priscol. The depressor amines lose whatever pressor action they may possess but retain their characteristic depressor action.

Priscol has great value as a physiological and pharmacological tool. By blocking the vasoconstricting actions of sympathomimetic substances, their vasodilating effects are unmasked and therefore become apparent. In the past most of the studies on the correlation of chemical structure with pharmacological activity of the sympathomimetics have been on comparative pressor activities. It is becoming more apparent that the sympathomimetic substances

are also potential vasodilators and that this action should also be considered in comparative studies.

Because Priscol has so many different pharmacological actions it must be used with care therapeutically. When used as a vasodilator its side actions such as cardiac stimulation, gastro-intestinal and uterine stimulation should be kept in mind. Undesirable increase in blood pressure or gastro-intestinal distress may result. The effectiveness of Priscol as an adrenolytic agent in humans has not yet been demonstrated.

CONCLUSIONS

1. Benzyl imidiazoline (Priscol) acts primarily as a sympathomimetic agent. Its chief sympathomimetic effects are: 1) peripheral vasodilation, 2) cardiac stimulation, 3) coronary vasodilation, 4) increasing cardiac output, and 5) some inhibition of the gastro-intestinal musculature. The effect of Priscol on the arterial pressure of dogs and cats (and probably humans) represents the algebraic summation of two opposing actions; peripheral vasodilation and increased cardiac output. In many dogs a pressor response results and in most cats a depressor response is produced.

2. Priscol also possesses some histamine-like properties. These are: 1) coronary constriction in the rabbit, 2) increased arterial pressure in the rabbit, and 3) stimulation of the isolated gut and the isolated or intact uterus. These actions are not prevented by atropine.

3. Priscol also produces some acetylcholine-like effects. These are: 1) cardiac slowing in rabbits, and 2) stimulation of the intact gastro-intestinal musculature. These effects are prevented by atropine.

4. Priscol is also an effective sympathomimetic anti-pressor and adrenolytic agent. It selectively blocks the pressor action of these substances allowing any masked depressor action to become apparent. The depressor amines lose only what little pressor action they may possess and retain unchanged their characteristic depressor actions. Priscol also blocks the excitant action of epinephrine on the uterus thereby unmasking the uterine inhibitory action of epinephrine.

This work was supported in part by a grant from Ciba Pharmaceutical Products, Inc. The authors wish to thank the following for supplying the various drugs: Bilhuber-knoll Corp. (EA-1), Wm. S. Merrell Co. (Vonedrine), Lederle Laboratories (Heparin), Eli Lilly Co. (Renin and Angiotonin), Sharpe and Dohme (Propadrine), Smith, Kline and French (Paredrine), Frederick Stearns and Co. (Neosynephrine, Sympatol, #277 and #1024), and Winthrop Chem. Co. (Ethyl nor-suprarenin).

REFERENCES

1. Weitzmann, G.: Munch. med. Wschr., 88: 99, 1941.
2. Lindquist, T.: Acta med. scand., 113: 83, 1943.
3. Hartmann, M., and Isler, H.: Arch. exp. Path. u. Pharma., 192: 141, 1939.

4. Hermann, H., Jourdon, F., and Bonnet, V.: Compt. rend. Soc. Biol., 135: 1653, 1941.
5. Chess, D., and Yonkman, F. F.: Fed. Proc., 4: 114, 1945.
6. Chess, D., and Yonkman, F. F.: Proc. Soc. Exp. Biol. and Med., 61: 127, 1946.
7. Bertazzi, C. G., and DeGennaro, A.: Riv. Pat. Sper., 29: 49, 1942.
8. Yonkman, F. F., Hays, H. W., Cameron, A., Pellet, E., and Hansen, N.: Fed. Proc., 5: 216, 1946.
9. Meier, R., and Meyer, R.: Schweiz. med. Wschr., 71: 1206, 1941.
10. Hamilton, W. F., Brewer, G., and Brotman, I.: Am. J. Physiol., 107: 427, 1934.
11. Soskin, S., Priest, W. S., and Schutz, W. J.: Am. J. Physiol., 108: 107, 1934.
12. Gregg, D. E., Shipley, R. E., Eckstein, R. W., Rotta, A., and Wearn, J. T.: Proc. Soc. Exp. Biol. and Med., 49: 267, 1942.
13. Frank, Otto: Ztschr. fur Biol., 37: 483, 1899.
14. McWilliam, J. A.: Proc. Royal Soc., 70: 109, 1902.
15. Hamilton, W. F.: Am. J. Physiol., 141: 235, 1944.
16. Remington, J. W., and Hamilton, W. F.: Am. J. Physiol., 148: 14, 1947.
17. Salant, W., and Parkins, W. M.: This Journal, 44: 369, 1932.
18. Woodbury, R. A., and Abreu, B. E.: Am. J. Obst. and Gyn., 48: 706, 1944.
19. Goodman, L., and Gilman, A.: The Pharmacological Basis of Therapeutics. The MacMillan Co., New York, 1941.
20. Craver, B. N., Chase, H. H., and Yonkman, F. F.: This Journal, 82: 275, 1944.
21. Warren, M. R., Marsh, D. G., Thompson, C. R., Shelton, R. S., and Becker, T. J.: This Journal, 79: 187, 1943.
22. Cameron, W. M., Whitsell, L. J., Crismon, J. M., and Tainter, M. L.: This Journal, 63: 340, 1938.
23. Schaumann, O.: Arch. exp. Path. u. Pharma.: 160: 127, 1931.
24. Lands, A. M., Rickards, E., and Nash, V. L.: Fed. Proc., 5: 60, 1946.
25. Scholz, C. R.: Ind. Eng. Chem., 37: 120, 1945.
26. Ahlquist, R. P., and Woodbury, R. A.: Fed. Proc. 5: 161, 1946.

FACTORS INFLUENCING THE TOXICITY OF SYMPATHOMIMETIC AMINES TO SOLITARY MICE

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Received for publication May 13, 1946

Reports on the toxicity of sympathomimetic amines contain several observations on the variable toxicity of these drugs to small laboratory animals and to mice in particular. "According to Maire and Franschine the LD_{50} of amphetamine is 280 mg /kg.; according to Chakravarti it is 155 mg /kg. and according to Halpern it is 25 mg./kg." (1). Other authors quote figures to show a similar though smaller variation in the toxicity of amphetamine and *d*-desoxyephedrine to rats and rabbits

It was probable that this variable toxicity was due to a complex set of factors. An investigation was, therefore, planned on this assumption.

Routine toxicity determinations in these laboratories are carried out in boxes containing ten mice at 80°F. Under these conditions mice treated with doses of amphetamine sulphate exhibited great excitement As the presence of other mice is, however, the most potent single factor influencing the toxicity, the effect of aggregation upon the toxicity of sympathomimetic amines has been made the subject of an earlier paper, (2), and only brief mention of the influence of one mouse on another will be made here. The following studies are, therefore, concerned entirely with solitary mice.

Three main considerations have determined the scope of this investigation. First, the need to determine the subcutaneous toxicity of amphetamine sulphate accurately for our own purposes Since reports in the literature make it clear that the subcutaneous route has given the greatest variability in the hands of different workers, this investigation was restricted to that route Secondly, chemically related amines mentioned in the literature have been included. As a result, the findings of workers in other laboratories have been explained, and certain interesting properties of these compounds have been brought to light. Thirdly, the relative importance of the different factors have been quantitatively assessed only where this might be expected to assist others to check our results, or where such assessments by revealing the relative importance of the different major factors, help an understanding of the mode of action of the drugs

METHODS The hydrochlorides of methedrine and ephedrine and the sulphate of amphetamine have been used for this work All of these were dissolved in redistilled water and single injections were made with a number 14 "Record" needle into the subcutaneous space behind the head

Comparisons were made in this way of the toxicities of the different substances under various conditions The substances were tested contemporaneously At least two re-

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sponses were obtained on the dosage mortality curve close to the median response, with a minimum of ten mice at each dose level. The number of mice involved, therefore, in any comparison was at least forty, and in some instances as many as 120 mice were used in a single comparison. The regression co-efficient of probit percentage mortality on log dose and the LD_{50} were calculated by the method of least squares and the values were checked by plotting the equation to the line found by calculation. Sometimes it was considered desirable to use more animals. Weighted values were obtained for the probits whenever the distribution of the response was unsymmetrical about 5.0 or when 0% or 100% responses were included in the calculations. In calculating the values of LD_{50} and of b (regression co-efficient), we have followed substantially the method of Bliss, (3). The values of s^2 and of s^2_b consequently represent the variances of a single observation and of the regression co-efficient respectively. They are the squares of the corresponding logarithmic standard errors. They are included in the tables to enable others to calculate the significance of differences found, where these may be considered to reveal points of interest. The limits of error for any value of LD_{50} are found by multiplying s by the appropriate factor (e.g. 1.96 for $P = 0.95$). In Table IX the values given under the column "Mean" have been got in the usual manner, by weighting the separate results with their variance reciprocals. In the column "Percentage Error," we have, following the procedure advocated by Bliss,

TABLE I

Comparison of the toxicity of amphetamine sulphate to male and female mice

Mortality in groups of 10 mice isolated in transparent jars (room temperature 60 F.).

DOSE	"SWISS"		GFF		A2	
	♂	♀	♂	♀	♂	♀
mg./kg.						
150	0	1	6	6	2	2
180	2	3	—	—	—	—

(3), calculating the limits of error from the value of s and the appropriate factor, but have expressed these as simple percentage deviations from the mean, instead of using the more strictly correct logarithmic deviations.

RESULTS. I. *Factors influencing the internal environment of the mouse.*

(a) *Feeding.* This has been standardised for all tests by removing the food from a box of fifty mice two to three hours before the beginning of the test. This procedure allows time for food in the stomach to be digested without withholding food long enough to bring on starvation changes, which begin between four and six hours later.

(b) *Sex.* In order to facilitate the subsequent stages of the investigation, the absence of any sex difference in sensitivity to the toxic effects of amphetamine sulphate was demonstrated for solitary mice of the pure line (Strong A2), of the inbred fawn strain (GFF) and of the imported "strain" (Swiss). See Table I.

(c) *Body weight and strain differences.* The results of the comparisons made with solitary mice of the three strains, with body weights between 16 to 20 grams and between 22 and 26 grams, are shown in Table II. It will be seen that an increase in body weight of all three strains is accompanied by an increased susceptibility to amphetamine poisoning and that the Swiss strain is less susceptible to the toxic influence of this drug than are the FF and A2 strains. This coincides with our observation that animals of this strain are noticeably more

active and aggressive and behave in a more excitable manner when kept together in large numbers than do those of the two inbred strains. This table also contains a comparison between male and female FF mice of body weights 16 to 20 grams, which confirms the evidence, presented earlier (Table I), that there is no difference between the susceptibilities of male and female FF mice.

(d) *Hydration.* The hydration of the body is known to affect the activity of the higher nervous centres, and it was therefore considered essential to obtain

TABLE II

Influence of sex, body-weight and strain difference on toxicity of amphetamine

STRAIN	"SWISS"		CFF			A2	
SEX	♂		♂	♀	♂	♂	
Body-weight (gms.)	16–20	22–26	16–20	16–20	22–26	16–20	22–26
LD50 (mg./kg.)	210.2	190.6	146.2	142.4	119.6	164.0	113.5
Variance (s^2)	0.000667	0.0006466	0.001453	0.00026618	0.006432	0.0007046	0.00142
Regression co-efficient (b)	10.8	10.4	6.3	4.00	5.6	5.51	10.6
Variance (s^2_b)	40.01	11.68	4.715	0.6225	8.654	1.788	8.1

TABLE III

Effect of hydration on toxicity

SUBSTANCE	TEMPERATURE / Volume of water/kg. injected with drug	LD 50 (mg./kg.) 5 ml.	LD 50 (mg./kg.) 50 ml.	REGRESSION CO-EFFICIENT (b) 5 ml.	REGRESSION CO-EFFICIENT (b) 50 ml.
Amphetamine	80°F.	39.5 (0.00300)	67.8 (0.00270)	7.95 (6.7)	7.15 (11.1)
	60°F.	195.7 (0.00127)	167.4 (0.001358)	15.84 (158.1)	21.33 (58.987)
Ephedrine	80°F.	171.2 (0.06148)	200.9 (0.05483)	2.57 (2.891)	1.74 (3.639)

Figures for variance, s^2, are given in parentheses.

information on the effects of varying the quantity of water in which the dose was administered. The results are shown in Table III. It will be seen that at 80°F. increasing the extent of hydration decreases the toxicity. The explanation for this is not clear, but the effects are sufficiently important at 80°F. to add considerably to the difficulty of obtaining consistent figures for the toxicity of amphetamine. The effect, if any, on the toxicity of ephedrine is not sufficient to be significant for most laboratory experiments.

II. *Specific relationship of the mouse with its environment.*

(a) *The influence of the presence of other mice.* This factor increases in importance as the number of mice aggregated together is increased, (2). Under conditions of confinement in groups, normally practised in pharmacological laboratories, this factor has the greatest effect on the toxicity.

(b) *The effect of confinement on the toxicity for solitary mice.* Under this heading two factors had to be considered, the first being the area in which the animal is free to move. This feature of the environment was shown to influence the toxicity of amphetamine and ephedrine for solitary mice by comparing the toxicity in opaque jars, the area of which was 50 cm², and in metal boxes, the area of which was 590 cm². Table IV shows that increasing the area decreases the susceptibility to the toxic influences of these drugs.

The second aspect of confinement concerns the transparency of the boundary walls. Its effect is found to be non-existent for solitary animals confined in a small area. The results of comparing the effect of transparent and opaque jars on the toxicity to solitary mice are shown in Table V.

(c) *The effect of sound.* Noises occurring in the laboratory and the squeaks of other mice activated with ephedrine and amphetamine were found responsible

TABLE IV

Effect of area on toxicity to solitary mice

SUBSTANCE	AREA	LD50	P VALUE FOR DIFFERENCE	REGRESSION CO-EFFICIENT (b)
	sq. cms.	*mg./kg.*		
Amphetamine	50	55.0	<0.01	4.4 (0.22468)
	590	111.3		5.1 (7.6200)
Ephedrine	50	242.4	<0.05 >0.02	2.3 (0.16082)
	590	550.3		2.9 (3.7640)

Figures for variance, s^2, are given in parentheses.

for violent outbursts of activity shown by groups of mice receiving these drugs. Careful observation, moreover, showed that sudden intermittent noises repeatedly produced alarm reactions in isolated mice that had received amphetamine or ephedrine. Intermittent noises of the type encountered in the laboratory were therefore imitated by hitting a metal mouse box at intervals for four hours after the injection of the drug. This type of stimulus significantly increased the mortality of isolated mice in jars given toxic doses of amphetamine. Those receiving ephedrine responded in the same way, though to a less degree. Table VI shows that the influence of sound on the toxicity of ephedrine to solitary mice was not significant and also that this effect is absent in the "benzedrinised" mice when they are confined in a space sufficient for freedom of movement.

III. *General environmental conditions.*

(a) *Light.* The intensity of illumination throughout the previous experiments had been constant at approximately 12 foot candles. Since, however, under normal conditions the intensity of illumination varies considerably throughout the period of a test, and since experiments reported below have shown that a

number of factors exert their influence by operating within the visual field of the animal, changes in intensity of the light might affect the mortality. Accordingly, a comparison was made between solitary mice at illuminations of the usual intensity, approximately 12 foot candles, and others at 1/100th of this (0.125 foot candles). The results given in Table VII show that the intensity of light is of no relevance.

TABLE V

Effect of opacity of surrounding walls on mortality of solitary mice at 80 F.

SUBSTANCE	DOSE	NO. IN GROUP	MORTALITY IN	
			Opaque jars	Transparent jars
	mg./kg.			
Amphetamine .. .	100	25	19	19
Ephedrine.. . .	200	20	8	7

TABLE VI

Effect of Noise on Mortality of Solitary Mice

SUBSTANCE	DOSE	CONFINED IN	NO. IN GROUP	SILENT	WITH NOISE	P VALUE
	mg./kg.					
Amphetamine	64	jars	40	16	25	<.04
	80	boxes	20	8	7	not calculated
Ephedrine	200	jars	40	12	16	>.05
	400	boxes	20	5	7	not calculated

TABLE VII

Effect of intensity of illumination on mortality

SUBSTANCE	DOSE	NO OF MICE	MORTALITY UNDER DIFFERENT ILLUMINATION (FOOT CANDLES)		P VALUE
			12 6	0 025	
	mg./kg				
Amphetamine.	100	20	14	14	Not calculated
Ephedrine	400	20	11	6	>.05

(b) *Temperature.* Other workers, (4), have recently demonstrated the influence of temperature on the toxicity of different drugs, a fact that we have amply confirmed in these laboratories with a number of drugs of different types. The effect of temperature on the toxicity of the amines is set out in Table VII; it is clear that the temperature of the surroundings has a profound influence on the toxicity and mode of action of these drugs. Amphetamine, methedrine and ephedrine have higher toxicities at 80°F. than at 60°F.

IV. *Comparison of relative toxicities.* The existence of various factors influencing the toxicity of sympathomimetic amines causes their relative toxicities

to differ according to the conditions under which they are determined. Many of the conditions of our final comparisons are arbitrary, such as the amount of water injected, the strain of mouse and the range of body-weights within which the mice were selected for test. Nevertheless, it was thought worth while to make the comparisons under conditions in which the central excitation is reduced to a minimum and the temperature is maintained in the neighbourhood of the optimal for mice (80°F.). At this temperature a balance between heat output and heat loss is achieved and the mice, therefore, need make no physiological adjustment to maintain a constant temperature. By these two means known conditions of stress have been reduced to a minimum.

The results (Table IX)[1] show close agreement and demonstrate that the most important influences affecting the toxicity of the amines to mice have been revealed.

TABLE VIII

Effect of temperature on toxicity

SUBSTANCE	LD50 (MG./KG. AT ROOM TEMPERATURE OF:		REGRESSION CO-EFFICIENT (b) AT ROOM TEMPERATURES OF:	
	80°F.	60°F.	80°F.	60°F.
Amphetamine	90.0 (0.00141)	197.0 (0.00008683)	10.26 (21.6)	13.18 (2.371)
Methedrine	33.2 (0.01114)	111.0 (0.00002862)	8.57 (12.23)	26.6 (2.437)
Ephedrine	477.1 (0.01807)	565.0 (0.0001540)	7.84 (15.33)	26.6 (8.649)

Figures for variance, s^2, are given in parentheses.

Discussion. It has been shown that the toxicity of amphetamine and, to a smaller extent, the toxicity of the other amines is influenced by a large number of factors. Some of these are now generally recognised as important factors to be controlled in pharmacological studies. Feeding, hydration, sex, body-weight and external temperature, for example, influence the action of many drugs. How they influence the toxicity of any particular drug is, however, not understood, except in a few instances.

As far as the evidence presented here shows, the importance of any modifying set of circumstances is always greatest for amphetamine, and less for methedrine and ephedrine, in that order. This is consistent with the hypothesis that the factors which influence the toxicity of these amines do so by altering the excitability of the central nervous system, since the action of ephedrine or methedrine on the central nervous system is known to be less than that of amphetamine.

The most striking feature of amphetamine is the variety of factors to which

[1] Further confirmation of this fact is provided by two comparisons of the relative toxicities which were made before all the conditions affecting the toxicity had been discovered. The results of these comparisons did not show the degree of agreement shown in Table IX.

the mouse becomes sensitive when under the influence of high doses. Since, moreover, the central nervous stimulation is the most predominant pharmacological action of this drug, it is probable that the number of factors influencing its toxicity is a manifestation of the increased sensitivity to stimuli induced by its action on the central nervous system.

It was demonstrated by Gunn and Gurd, (5), that the presence of other mice induced excitation in mice receiving high doses of amphetamine and allied drugs, but that the solitary mouse receiving large doses of amphetamine remained unexcited although exhibiting increased spontaneous activity. A more detailed description of the behaviour of solitary mice and groups of mice receiving large doses of amphetamine has been the subject of other publications. The essential feature of these amines is that, unlike other central nervous stimulants, they induce in the mouse a state of excitability rather than of excitement. Lethal doses almost invariably kill the solitary mouse as a result of violent convulsions,

TABLE IX

Relative toxicity of sympathomimetic amines to inbred mice under controlled conditions

SUBSTANCE	LD 50 MG./KG.					REGRESSION CO-EFFICIENT (b)				
	Date of Expt.			Mean	Percentage error (P = 0.05)	Date of Expt.			Mean	Percentage error (P = 0.05)
	10/10/44	23/10/44	27/12/44			10/10/44	23/10/44	27/12/44		
Amphetamine	89.54	74.80	74.14	79.80	±12.2	8.56	7.14	8.50	8.21	±36.6
Ephedrine	238.58	308.90	275.81	276.97	±27.4	2.88	4.46	1.44	2.13	±62.8
Adrenaline	4.58	4.75	3.72	3.86	±19.8	6.00	5.21	7.47	6.69	±36.2
Methedrine	15.85	21.87	20.85	20.70	±32.0	4.02	2.66	2.72	3.01	±49.0

Conditions of experiment: Solitary GFF mice, weighing 18 to 22 grams, isolated in silence in jars at 80 F., receiving the total dose in 20 mls. of H_2O per kg. body weight.

which, therefore, may appear when the excitation in the central nervous system reaches a critical level. Doses themselves not lethal may thus become so as a result of external factors raising the excitation to a critical level.

In view of all the factors altering the toxicity of these amines, it was thought probable that the excitation of the central nervous system may be increased by afferent impulses or decreased by efferent impulses during the action of these drugs. In this way, the number of animals reaching a critical level of excitation at which lethal convulsions occur is altered by a variety of environmental factors in mice receiving near lethal doses of amphetamine or related amines. Factors that increase afferent impulses would thus be expected to increase the toxicity and efferent discharges from the central nervous system to decrease it.

The effect of sound may thus be one means of increasing the central nervous excitation by increasing the afferent impulses along the auditory nerve and our results may be analogous to the effect of sound in inducing convulsive seizures in *Peromyscus* mice reported by Sumner, (6), to be due to a heritable factor; he found that some mice of this species were thrown into convulsions by certain types of sound. Similar, often lethal, convulsions were brought on by sound in solitary mice receiving large doses of these amines.

Freedom of movement, allowed by increasing the area in which the solitary mouse is free to move, may mean that the central nervous excitation is relieved by motor impulses passing to the muscles during movement round the box. This activity would also provide a means of relieving the central nervous excitation in mice subjected to repeated auditory stimulation and may explain why noise does not influence the solitary mouse which is free to move, but does affect the mouse confined in a small space (see Table VI).

The influence of environmental temperature on the response of mice to the toxic effects of these amines may also be due to the increase in the rate of discharge with rising body temperature, which Gellhorn, (7), suggests occurs in all parts of the brain. Conversely, pilo-motor impulses in shivering may relieve the central nervous excitation and so reduce the toxicity of these drugs at low temperature. This suggestion is made more likely by the work of Warren and Werner, (8),[2] who have found that amphetamine and ephedrine increase the body temperature of mice.

SUMMARY

1. Factors which influence the toxicity of sympathomimetic amines to solitary mice have been described. These include hydration, sex, strain differences, body-weight, external temperature, degree of confinement and sound.

2. A detailed investigation of the factors influencing the toxicity of amphetamine sulphate has been made. The effect of some of the relevant factors influencing the toxicities of ephedrine hydrochloride and methedrine hydrochloride have also been studied.

3. It is suggested that the sensitivity to the various factors induced by these substances is a function of their capacity to stimulate the central nervous system, and that a variety of factors modify the toxicity by altering the degree of central nervous excitation or its opportunities of discharge.

4. When the disturbing factors are controlled, it has been found possible to determine the toxicity of these drugs with the degree of accuracy usually encountered in biological estimations.

Acknowledgements. My thanks are due to P. Dirnhuber for valuable assistance in the statistical treatment of results, to H. Lobstein for technical assistance and to Mr. A. L. Bacharach for advice and criticism in preparing this paper and the earlier one (2).

REFERENCES

1. Gunther, B.: *J. Pharmacol.*, 76, 375, 1942.
2. Chance, M. R. A.: *J. Pharmacol.*, 87, 214, 1946.
3. Bliss, C. I.: Quart. J. Pharm. and Pharmacol., 11, 192, 1938.
4. Chen, K. K., Anderson, R. C., Steldt, F. A., and Mills, C. A., *J. Pharmacol.*, 79, 127, 1943.
5. Gunn, J. A., and Gurd, M. R., *J. Physiol.*, 97, 453, 1940.
6. Sumner, F. B.: Bibl. Genet., (Lpz) 9, 1, 1932.
7. Gellhorn, E.: Autonomic Regulations (New York) 1943.
8. Warren, M. R., and Werner, H. W.: J. Pharmacol., 86, 280, 1946.

[2] This work was published after our investigation was completed.

THE PHARMACOLOGY OF VASODEPRESSOR COMPOUNDS STRUCTURALLY RELATED TO THE SYMPATHOMIMETIC AMINES

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Received for publication November 27, 1946

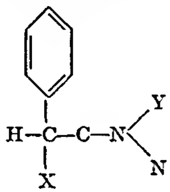
Konzett (1) has described the pharmacology of several N-alkyl homologues of epinephrine and reports that some of these, in suitable dose, cause a marked fall in blood pressure, although they retain many of the other pharmacologic actions of epinephrine. He also mentions the isopropyl homologues of 'Sympatol' (1-(p-hydroxyphenyl)-2-isopropylaminoethanol) and 'Neo-Synephrine' (1-m-(hydroxyphenyl)-2-isopropylaminoethanol), but does not describe their effect on blood pressure. We have carried out in this Laboratory an investigation of the pharmacologic actions of these two latter substances and of various other structurally related compounds. The synthesis of some of these compounds has been described by Corrigan, Langerman and Moore (2). The pharmacologic results obtained are described in this communication.

1. Effect on blood pressure. Carotid blood pressure was determined in dogs anesthetized with sodium pentobarbital. All injections were made into the exposed femoral vein. Average results obtained are shown in table 1. It should be noted that tyramine (1-(p-hydroxyphenyl)-2-aminoethane) is the most active pressor compound of the series shown in this table. The addition of the alcoholic hydroxyl to give 1-(p-hydroxyphenyl)-2-aminoethanol (No. 582) results in a marked reduction in pressor potency. Within the series reported here, subsequent alkyl substitution on the nitrogen leads to a further reduction in pressor potency, and, with the substitution of groups larger than methyl (table 1), the resultant compounds produce a depressor effect. However, the amount and duration of the fall in blood pressure varies greatly with these higher homologues. On the basis of their effect on blood pressure, they may be divided into two groups: 1) those containing one or more methyl groups attached to the first carbon of the N-alkyl group (Nos. 573, 277, 651 and 661), and, 2) those containing groups larger than methyl attached to the first carbon of the N-alkyl group (Nos. 579, 570 and 643). This second group causes a transient fall in blood pressure similar to that produced by a large number of structurally diverse amines. The first group, on the other hand, induces a marked and very prolonged reduction in blood pressure (fig. 1). This effect appears to be greatest with Nos. 277 and 661 and least with No. 859. Comparison of results obtained with Nos. 859 and 832 suggest that the alcoholic hydroxyl is importantly concerned with this action (table 2). The presence of a phenolic hydroxyl in either the meta- (No. 539) or para- (No. 277) position increased this action (table 1).

The pharmacologic action of the compounds in this first group can best be illustrated by No. 277, inasmuch as other members of the group cause similar responses. Repeated intravenous administration of small doses of No. 277 (0.05–0.1 mgm./kgm.) leads to a progressive fall in mean arterial pressure to about 50 or 60 per cent of the preinjection level. After this pressure has been reached, the injection of additional amounts of the compound is without further effect on blood pressure. The injection of as much as 1.0 or 2.0 mgm./kgm.

TABLE 1

Effect of compounds structurally related to tyramine on the blood pressure of the dog



COMPOUND	PHENOLIC OH	X	Y	DOSE	CHANGE IN B.P.	DURATION	TOXICITY	
							Approx. LD 50†	Number of mice
				mgm./kgm.	mm. Hg		mgm./kgm.	
Tyramine	para	H	H	0.18	+60	5 min.		
582	para	OH	H	0.21	+46	5–10 min.	600	50
'Sympatol'	para	OH	methyl	0.46	+45	5–10 min.	1000	34
573	para	OH	ethyl	0.50	−30	Prolonged	600	74
579	para	OH	n-propyl	0.50	−19	Transient	300	34
277	para	OH	isopropyl	0.20	−35	Prolonged		
				0.50	−63	Several hours	370	84
570	para	OH	n-butyl	0.50	−21	Transient	150	18
661*	para	OH	sec.-butyl	0.25	−26	Prolonged		
651	para	OH	tert.-butyl	0.25	−20	Prolonged		
				0.50	−60	Prolonged	250	28
643	para	OH	isobutyl	0.20	−4	Transient	220	37
539	meta	OH	isopropyl	0.30	−30	Prolonged	320	44

* The diastereoisomer racemates were separated. Data obtained with the more active member are shown here.

† Albino mice were used. The compounds were injected intraperitoneally.

in a single dose produces a large fall in blood pressure and subsequent injections may cause little or no further change. The intravenous injection of large amounts (5.0–10.0 mgm./kgm.) does not cause circulatory collapse. The observed reduction in blood pressure is brought about by vasodilatation in both the cutaneous and splanchnic areas. Following intravenous administration of No. 277 there is an increase in splanchnic volume (as recorded by the intestinal oncometer). Also, it may be noted that unpigmented skin areas become very pink with the onset of the fall in blood pressure.

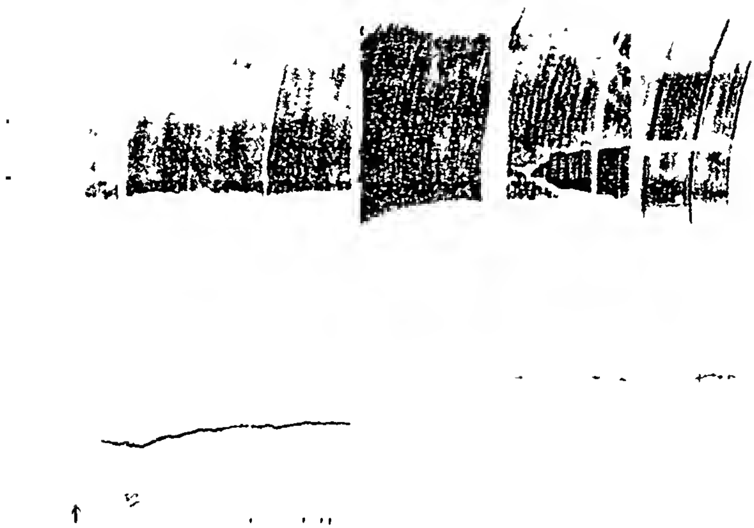
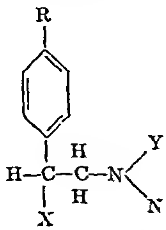


FIG. 1. EFFECT OF INTRAVENOUSLY INJECTED NO. 277 ON THE BLOOD PRESSURE OF THE DOG

From above downward is recorded respiration, blood pressure and time (in 10 seconds). The individual segments record: 1) injection, 2) 34 minutes, 3) 50 minutes, 4) 107 minutes after injection. No. 277, 0.5 mgm./kgm., was injected at the arrow.

TABLE 2

Effect of structural changes in the sympathomimetic nucleus on depressor action in the dog



COMPOUND	R	X	Y	DOSE	CHANGE IN B.P.	DURATION
				mgm./kgm.	*mm. Hg*	
126	H	H	ethyl	0.45	+3	Transient
573	OH	OH	ethyl	0.50	−30	Prolonged
832	H	H	isopropyl	0.50	−15	Transient
859*	H	OH	isopropyl	0.50	−36	Prolonged (½–2 hrs.)
277	OH	OH	isopropyl	0.50	−63	Several hours

* LD 50, intraperitoneal albino mice, 180 mgm./kgm. (49 mice).

In several experiments, No. 277 was injected intramuscularly into unanesthetized dogs. Except for a more gradual onset in the fall in pressure, the results (fig. 2) are essentially the same as those described following intravenous administration. In some experiments on anesthetized dogs, No. 277 was injected directly into a loop of the small intestine. The rapid onset of the fall in blood pressure observed indicates a rapid absorption from this organ (fig. 3). In several instances, No. 277 in aqueous solution was put directly into the stomach of unanesthetized dogs by intubation. A reduction of blood pressure with an increase in pulse rate was detected within 5 to 10 minutes and these changes

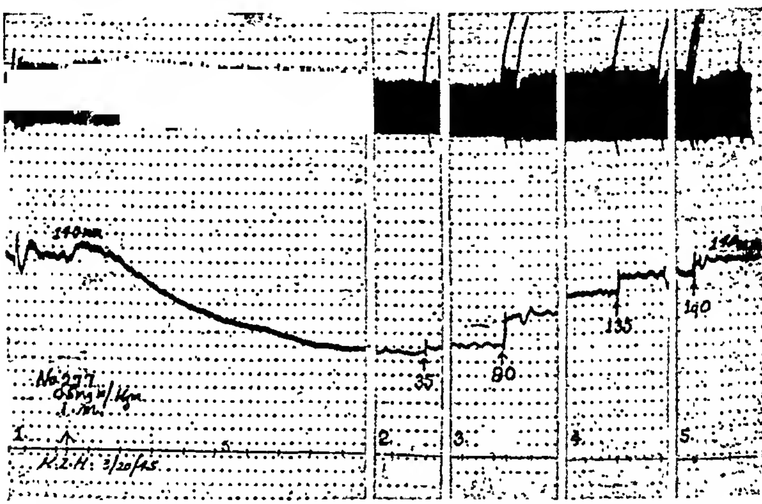


FIG. 2. EFFECT OF INTRAMUSCULARLY INJECTED NO. 277 ON THE BLOOD PRESSURE OF THE DOG

From above downward is recorded respiration, blood pressure and time (in minutes). The numbers under the blood pressure tracing indicate the total time in minutes. No. 277, 0.5 mgm./kgm., was injected at the arrow.

were maximal within 20 to 30 minutes (table 3). The effects lasted for several hours. At no time did the dogs show any signs of discomfort.

The effect on the action of the heart was determined in frogs, rabbits and dogs. With frogs, the brain and spinal cord were pithed and a cannula inserted into the sinus venosus. The truncus arteriosus or carotid arch was cut and the perfusion fluid allowed to drain away. All drugs were made up in frog Ringer solution and injected into the perfusion stream near the sinus cannula. With this preparation, 0.005 mgm. of epinephrine caused a prompt increase in both rate and amplitude that lasted about one minute. No. 277, in doses up to 1 mgm., either had no effect or caused a reduction of rate and amplitude that lasted for one to several minutes.

Isolated rabbit hearts were perfused according to the method of Langendorff. In contrast to results with frogs, the injection of 1 microgram of No. 277 directly

into the perfusion stream near the heart caused a marked increase in rate and amplitude. In several experiments on dogs, myocardiographic recordings of

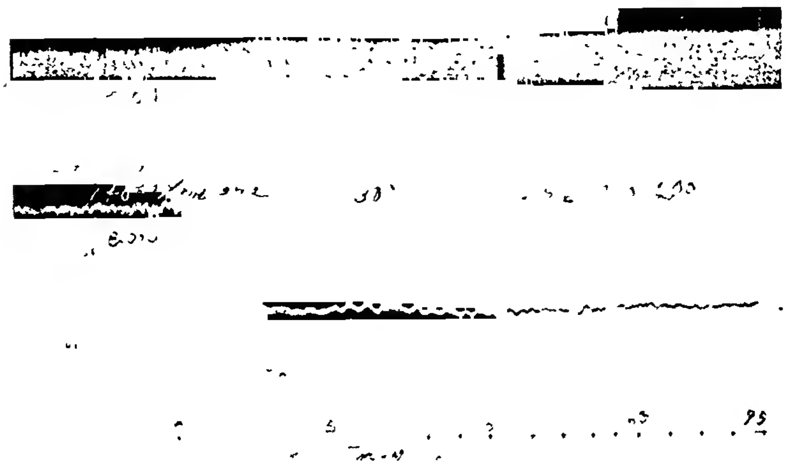


FIG. 3. EFFECT OF NO. 277 INJECTED INTO THE LUMEN OF THE SMALL INTESTINE, ON THE BLOOD PRESSURE OF THE DOG

From above downward is recorded respiration, heart rate, blood pressure and time (in minutes). The numbers on the time line indicate the total time in minutes. No. 277, 5.0 mgm./kgm., was placed in the small intestine at the arrow

TABLE 3

Effect of compound No. 277 on the blood pressure and pulse rate in unanesthetized dogs

MODE OF ADMIN	DOSE	INITIAL SYSTOLIC BLOOD PRESSURE/ INITIAL PULSE RATE	MAXIMUM CHANGE	DURATION OF ACTION
	mgm./kgm.	*mm Hg/beats per min*		
S.C.	0.25	150/68	−40/+112	B.P. normal within 4½ hrs. Pulse rate still greater than normal
S.C.	1.00	155/98	−60/+102	B.P. normal within 2½ hrs. Pulse rate still greater than normal
Oral	0.25	145/62	−45/+ 78	B.P normal within 4½ hrs. Pulse rate still greater than normal
Oral	0.50	195/70	−85/+ 74	More than 4 hours
Oral	1.00	125/64	−40/+146	B.P. normal within 5½ hrs. At this time pulse rate = 144
Oral	10.00	155/100	−49/+ 96	B.P 102, pulse rate 180, at end of 6½ hrs.

the left ventricle were made. With this preparation, No. 277, in intravenous doses of 0.1 mgm./kgm., caused a distinct increase in both rate and amplitude; the amplitude of contraction was greater than normal for about 5 minutes, while

the increased rate lasted for an hour or more. In an effort to prevent the reflex effects on the heart that result from a fall in mean arterial pressure, 0.01 milligram of 'New-Synephrine' HCl per kilogram was combined with 0.1 milligram of No. 277 per kilogram and the mixture was injected intravenously. This amount of 'Neo-Synephrine' has no demonstrable effect on the action of the heart but prevents the reduction in blood pressure from No. 277. In these experiments the increases in rate and amplitude were much the same as when No. 277 was injected alone. These results and those obtained with the isolated perfused rabbit heart suggest that the mammalian heart is strongly stimulated by No. 277.

2. Effect on the bronchioles. Isolated guinea pig lungs were perfused by the method of Sollman and von Oettingen, as modified by Tainter (3). Bronchoconstriction was induced by histamine acid phosphate (0.01 to 0.02 mgm.) injected directly into the perfusion system near the lung. The results obtained are shown in table 4. It should be noted that those compounds most active in reducing blood pressure are only moderately active as bronchodilators. They do not appear to be significantly more active than 'Sympatol'. Those compounds in which the N-alkyl group is unbranched are more active on the bronchioles. The N-ethyl derivative retains the pharmacologic properties of both series, being an active vasodepressor and bronchodilator substance.

TABLE 4

Bronchodilator action of compounds structurally related to tyramine

(Perfused isolated guinea pig lung)

COMPOUND	RESPONSE TO HISTAMINE	RESPONSE TO HISTAMINE + COMPOUND†
	cc./min.	*cc./min.*
582	45/26*	43/28
'Sympatol'	44/27	44/44
573	25/14	27/42
277	56/28	54/55
661	57/34	60/47
651	42/15	42/33
859	41/25	40/37
579	44/26	46/50
570	51/31	50/56
643	40/23	39/35

* Rate before histamine/rate after histamine.

† 10 mgm. of the experimental compound injected with the constricting dose of histamine.

3. Effect on the intestine. Action on the isolated segment of the small intestine was determined by the method of Magnus. Unstimulated segments of guinea pig ileum were used to determine the effect of these compounds on tonus and motility. The muscle bath had a capacity of 200 cc. and was filled and emptied twice between each determination. The results obtained are shown in table 5. These results are similar to those previously described for blood pressure. Compounds which cause a prolonged fall in blood pressure

also produce a marked reduction in tonus and motility when present in a concentration of 1:200,000. At this concentration, the n-propyl, n-butyl and isobutyl compounds are without effect. In a few experiments, motility of the rabbit jejunum, *in situ*, was recorded kymographically. Those drugs found to be most active in relaxing the isolated intestinal segments were also found to be quite active in relaxing intestinal tonus following their intravenous injection (0.5–1.0 mgm./kgm.).

4. Effect on the uterus. The activity of isolated segments of the non-pregnant rat, rabbit and guinea pig uteri was determined as described above for the intestine. Results obtained are shown in table 5. Epinephrine and

TABLE 5

Effect on the isolated intestine and uterus of compounds structurally related to tyramine

COMPOUND	ILEUM	UTERUS		
	Guinea pig	Rat	Rabbit	Guinea pig
β-Phenethylamine	E*	I	NA	E
Tyramine	NA	I	E	E
582		I	E	E
'Sympatol'	E	I	NA	NA
573	I	I	I	NA
277	I	I	I	NA
651	I	I	I	NA
859	I	I	I or NA	NA
579	NA	I	I or NA	NA
570	NA	I	E	NA
Epinephrine	I and E†	I	E	E

* Dilution of 1:200,000. This dilution was used for all compounds except epinephrine. E = excites, I = inhibits, NA = no action.

† I with dilutions of 1:10M to 1:40M. E with dilution of 1:1M.

tyramine inhibit motility of the rat uterus and increase either tonus or motility, or both, of the rabbit and guinea pig uterus. No. 277 either inhibits motility or is without effect (guinea pig uteri). There is no evidence of excitation in any instance. Similar results were obtained with other members of this series that cause a prolonged fall in blood pressure.

5. Toxicity. Comparative toxicity was determined by intraperitoneal injection into mice. The mice weighed 15 to 20 grams and were observed for 72 hours following injection. All animals were housed before and during the test in quarters maintained at 75°F., and at a constant humidity of 35 per cent. Results obtained are shown in table 1. Of the compounds investigated, the N-methyl derivative, 'Sympatol', was found to be least toxic. Those compounds containing an unbranched alkyl group on the nitrogen increase in toxicity with an increase in the length of the chain. Thus, No. 573 is more toxic than 'Sympatol', No. 579 is more toxic than No. 573, and No. 570 is most toxic. With compounds in which the alkyl group on the nitrogen is branched, isopropyl (No. 277) is least toxic. The tertiary butyl (No. 651) appears to be somewhat less toxic than the isobutyl derivative (No. 643). Toxicity of the secondary

butyl diastereoisomers could not be accurately determined since the compounds are insoluble in distilled water. The isobutyl compound (No. 643) is less toxic than the n-butyl derivative (No. 570).

The acute oral toxicity of Compound No. 277 was determined in 16 rats. Animals of both sexes weighing between 150 and 200 grams were given No. 277 in aqueous solution by intubation. Doses of 550–2000 mgm./kgm. were administered daily for 14 days. Only one death resulted (at 750 mgm./kgm.) from the administration of this compound. Examination revealed no histopathological changes. This compound has low oral toxicity in rats.

Six normal dogs were given No. 277 orally, 25 mgm./kgm. daily (6 days in each week) for periods of 8–12 weeks. The compound caused persistent low blood pressure and fast heart rates in all animals. However, examination of the blood revealed no significant change in the number of erythrocytes or of leucocytes, or in blood sugar, non-protein nitrogen, total serum protein or plasma chloride values. A complete pathological examination was made upon three of the above dogs, one animal was sacrificed at the end of 8, 10, and 12 weeks, respectively. No abnormalties were found in any organ.

Discussion. Numerous articles have reviewed the relationship between chemical structure and sympathomimetic action. Among the most recent is that of Hartung (4). However, the data presented do not provide as complete a correlation as might be desired. Data obtained with the same or similar compounds in various laboratories often differ. In order better to understand the relationship between chemical structure and pharmacologic action, we have determined the response to a reasonably complete series of compounds which may be considered as derivatives of tyramine. In this series, it is of interest to note that the addition of an alcoholic hydroxyl to the beta-carbon of the side chain and N-methylation both diminish the pressor potency of tyramine. 'Sympatol' (1-(p-hydroxyphenyl)-2-methylaminoethanol), which differs from epinephrine only in that it does not contain a second hydroxyl in the meta-position on the phenyl ring, is only $\frac{1}{4}$ to $\frac{1}{5}$ as pressor as tyramine and has about 1/316th the pressor activity of epinephrine.

This diminishing pressor potency takes on added significance when the pharmacology of the N-ethyl homologue is considered. This compound is predominantly depressor, does not stimulate the perfused frog heart, relaxes the guinea pig ileum, either relaxes or has no action on the uterus and dilates the perfused guinea pig bronchioles. Such actions would be expected to result if the inhibitory (sympathin I-mimetic) actions were retained with suppression of the excitatory component (sympathin E-mimetic). A comparison of various N-alkyl derivatives clearly indicates a specificity of action comparable to that shown by pressor sympathomimetic amines. Thus, the secondary beta-p-hydroxyphenylethanolamines, with the structure $-\mathrm{N}-\underset{\mathrm{CH_3}}{\overset{\mathrm{R}}{\mathrm{C}}}-\mathrm{R}$, behave like the N-ethyl derivative, with greatest activity being found with the isopropyl compound. The n-propyl, n-butyl and isobutyl derivatives are quite different in their behavior and pharmacologically resemble amines with widely varying

structures. These latter substances have only a transient effect on blood pressure and cause papaverine-like depression of smooth muscle, when present in high concentration.

The structure of the sympathomimetic nucleus likewise influences the results obtained. Thus, 1-phenyl-2-isopropylaminoethanol (No. 859) causes a prolonged fall in blood pressure. Our investigation leads us to postulate that, in the compounds studied, the addition of an hydroxyl to the ring, in either the meta- (No. 539) or para- (No. 277) position, increases the depressor activity. Chen Wu and Henriksen (5) have reported that both the N-ethyl and N-isopropyl homologues of 1-phenyl-2-ethylaminopropane is slightly pressor. We have found 1-(p-hydroxyphenyl)-2-isopropylaminopropanol to be depressor in dogs, but considerably weaker in action than 1-(p-hydroxyphenyl)-2-isopropylaminoethanol.

Acute toxicity data indicate that N-alkylation increases toxicity. In general, our results are in agreement with those obtained by Chen et al. (5) with ephedrine derivatives. However, 'Sympatol', a secondary methylamine, is a notable exception, in that it is the least toxic compound in the series. The N-ethyl homologue is only slightly more toxic than the primary amine. The branched chains are less toxic than the corresponding normal chains. Thus, n-propylamine is more toxic than the isopropylamine and n-butylamine more toxic than the tertiary and isobutylamines. The presence of an hydroxyl in the para-position on the ring diminishes toxicity significantly, compound No. 277 being about one-half as toxic as No. 859.

SUMMARY

1. The pressor potency of 1-(p-hydroxyphenyl)-2-aminoethane (tyramine) is diminished by the addition of an hydroxyl group to the beta-carbon and by the addition of a methyl group to the nitrogen.

2. The replacement of one of the hydrogens of the amino group of 1-(p-hydroxyphenyl)-2-aminoethanol by ethyl, isopropyl, secondary butyl or tertiary butyl groups results in compounds that are strongly depressor in action. Similar substitution by n-propyl, n-butyl and isobutyl groups results in compounds which exert only transient depressor action.

3. The presence of an alcoholic hydroxyl on the beta-carbon appears to be necessary for this action.

4. Those compounds which cause a prolonged fall in blood pressure are also most active in bringing about inhibition in those organs inhibited by epinephrine.

REFERENCES

1. Konzett, H.: Arch. f. exper. Pathol. u. Pharmakol., 197: 27, 1940; 197: 41, 1940.
2. Corrigan, J. R., Langerman, M. J., and Moore, M. L.: J. Am. Chem. Soc., 67: 1894, 1945.
3a. Sollmann, T., and von Oettingen, W. F.: Proc. Soc. Exper. Biol. Med., 25: 692, 1928.
3b. Tainter, M. L.: This Journal, 51: 371, 1934.
4. Hartung, W. H.: Indust. and Engin. Chem., 37: 126, 1945.
5. Chen, K. K., Wu, Chang-Keng and Henriksen, E.: This Journal, 36: 363, 1929.
6. Hauschild, F.: Arch. f. exper. Pathol. u. Pharmakol., 195: 647, 1940.

THE EFFECT OF PERFUSION THROUGH THE ISOLATED LIVER ON THE PROTHROMBIN ACTIVITY OF BLOOD FROM NORMAL AND DICUMAROL TREATED RATS

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Received for publication November 29, 1946

The necessity of the liver for the maintenance of the prothrombin activity of the blood has been clearly demonstrated by means of hepatectomy (1) the influence of hepatotoxic drugs (2, 3) and clinical observation in hepatic disease (4). The inference that prothrombin is actually formed in the liver seems well justified; nevertheless, the actual formation of prothrombin by liver tissue has not been demonstrated.

During the course of experiments concerning the mechanism of action of dicumarol it has been possible to demonstrate an increase in the prothrombin level following the perfusion of the isolated liver with blood deficient in prothrombin activity. The results of these studies constitute the material for this report.

MATERIALS AND METHODS. Stock white rats were used throughout the investigation. Eight mg. of dicumarol, suspended in a 1 per cent tragacanth emulsion, was administered per day by means of a rigid stomach tube. One or two doses were given and the animal was utilized at the end of 24 or 48 hours. Blood was obtained either by cardiac puncture or exsanguination through the abdominal aorta, being drawn into a syringe containing 3.8 per cent sodium citrate solution, the final mixture being in the proportion of one of citrate to nine of blood. Under ether anesthesia the liver for perfusion was exposed by a wide cruciate incision of the abdominal wall. The portal vein was exposed and the distal end ligated. A small glass cannula, connected to a flask of 0.85 per cent saline at 37°C., was inserted into the hepatic end of the portal vein and ligated in position. The liver was then washed with the normal saline to demonstrate the success of the cannulation and to remove the intrahepatic blood of the liver donating rat. This washing was repeated after the liver was removed from the rat carcass. Excess tissue was trimmed away and the liver, suspended by the ligature on the cannula, placed in the perfusion chamber.

Perfusion apparatus. From 50 to 60 cc. of pooled blood were placed in a reservoir connected to the liver cannula. Blood flow was by gravity from the reservoir through the liver, out the hepatic veins, and thence to the paraffinized floor of the chamber. Here it was pooled and then pumped back into the reservoir, where oxygen was bubbled through the blood. Perfusion pressure of between 18 and 22 cm. of water was maintained, with slight fluctuations due to the action of the pump. The reservoir and perfusion chamber were maintained at atmospheric pressure by means of a connecting airway which permitted free escape of the inflowing oxygen. The whole apparatus was suspended in a water bath maintained between 37° and 38°C.

Prothrombin determinations were made by the one stage method. Thromboplastin was prepared by the method of Link and his associates (5), then mixed with an equal volume of 0.025M $CaCl_2$. Plasma was mixed with equal parts of a fibrinogen solution prepared by the method of Jaques (6). Prothrombin time was determined to be the time of formation of a clot after the introduction of 0.2 ml. of the thromboplastin-calcium mixture into the plasma-fibrinogen mixture.

Preliminary experiments with diluted blood resulted in wide fluctuations of the pH of the perfusate during the course of the perfusion. Because of the marked effect of pH on the prothrombin determination (7) whole blood was used throughout and the pH of the samples determined routinely. At no time was pH less than 7.05 nor greater than 7.95, as determined by the Leeds and Northrup glass electrode pH meter. Prothrombin activity is not materially effected within this range (8).

RESULTS. *Control determinations.* Perfusion of normal whole pooled rat blood through an open cannula resulted in no significant change in the prothrombin level over a period of four hours (see table 1).

TABLE 1

Perfusion of pooled whole blood through an open cannula

The prothrombin times are recorded in seconds (average of three determinations)

	PERFUSION TIME IN MINUTES				
	0	60	120	180	240
Blood from normal rats					
#1	16.0	16.0	15.5	15.5	16.0
#2	17.0	16.5	16.0	16.0	17.0
#3	16.0	16.0	16.0	17.0	17.0
Blood from dicumarol treated rats					
#1	35.0	41.5	44.5	51.5	52.5
#2	44.0	52.0	60.5	64.5	66.0
#3	64.0	68.0	74.0	83.0	99.0

Perfusion of blood from dicumarol treated rats, with resulting low prothrombin activity, through an open cannula resulted in a prolongation of the prothrombin times by 35, 17, and 22 secs. Compared with the activity of the original plasma this represents a moderate decrease in prothrombin activity. Data from three such determinations are presented in table 1.

Perfusion of normal blood through the liver of a normal rat resulted in no significant change in the level of the prothrombin activity. Data from three such determinations are presented in table 2.

Perfusion of blood from dicumarol treated rats through the liver of a normal rat. The perfusion of blood from dicumarol treated rats through the liver of a normal rat resulted in a significant increase in the prothrombin activity of the perfused blood. As a rule the increase varied with the original prothrombin level. Almost all the change occurred within the first two hours. See table 2 and figure 2. Prothrombin times are plotted against perfusion times in figure 2 and may be compared with the control activities plotted in figure 1.

Perfusion of blood from dicumarol treated rats through the liver of a dicumarol treated rat. The perfusion of blood from dicumarol treated rats through the liver of a dicumarol treated rat resulted in a progressive, slight increase in prothrombin time. When compared with the increase in prothrombin time when

dicumarol blood is perfused through an open cannula, there is no significant difference. Data from three such determinations are presented in table 3 and should be compared with the results recorded in table 1.

Perfusion of normal blood through the liver of a dicumarol treated rat. The perfusion of normal blood through the liver of a dicumarol treated rat resulted in no change in the prothrombin activity (see table 3).

DISCUSSION. Perfusion of the liver is a highly artificial procedure. It has the advantage of restricting the results obtained to the organ involved. In the Vitamin K deficient animal the relatively prolonged latent period between the time of receiving the specific vitamin and the increase in the prothrombin activity makes it seem likely that prothrombin is formed gradually over a considerable period of time. For this reason it was felt that any perfusion must be capable of being continued with the same perfusate for a period of hours. This was effected by a system which recirculated the perfusate through the perfused organ. It is doubtful whether the liver under such conditions retains much semblance of normalcy after two hours of perfusion. Though the perfusion was continued for four hours, in no case was there a significant increase in prothrombin, after the second hour. No conclusion as to the reason for the cessation of the activity by the second hour is justified, since a multitude of events may have occurred.

TABLE 2

Perfusion of pooled whole blood through the liver of a normal rat

The prothrombin times are recorded in seconds (average of three determinations)

	PERFUSION TIME IN MINUTES				
	0	60	120	180	240
Blood from normal rats					
#1	19.5	17.5	19.5	17.5	18.0
#2	15.5	16.0	15.0	15.0	15.5
#3	17.5	17.0	17.0	18.0	18.0
Blood from dicumarol treated rats					
#1	41.5	31.0	30.0	25.0	25.5
#2	129.0	78.0	43.0	38.0	38.0
#3	74.5	34.5	29.0	29.0	29.0
#4	77.0	38.5	37.5	38.0	—
#5	57.0	33.0	32.5	31.5	31.5
#6	96.0	31.0	28.0	—	—
#7	134.0	32.5	30.0	39.5	29.5

Results with normal blood, both through an exposed cannular opening and through a normal liver, demonstrate no significant alteration in the prothrombin activity under the perfusion conditions.

However, when blood from dicumarol treated rats was perfused through the

open cannula (without a liver in the system) or through a liver from a dicumarol treated rat, a slight decrease in activity occurred. The decrease with a dicumarol

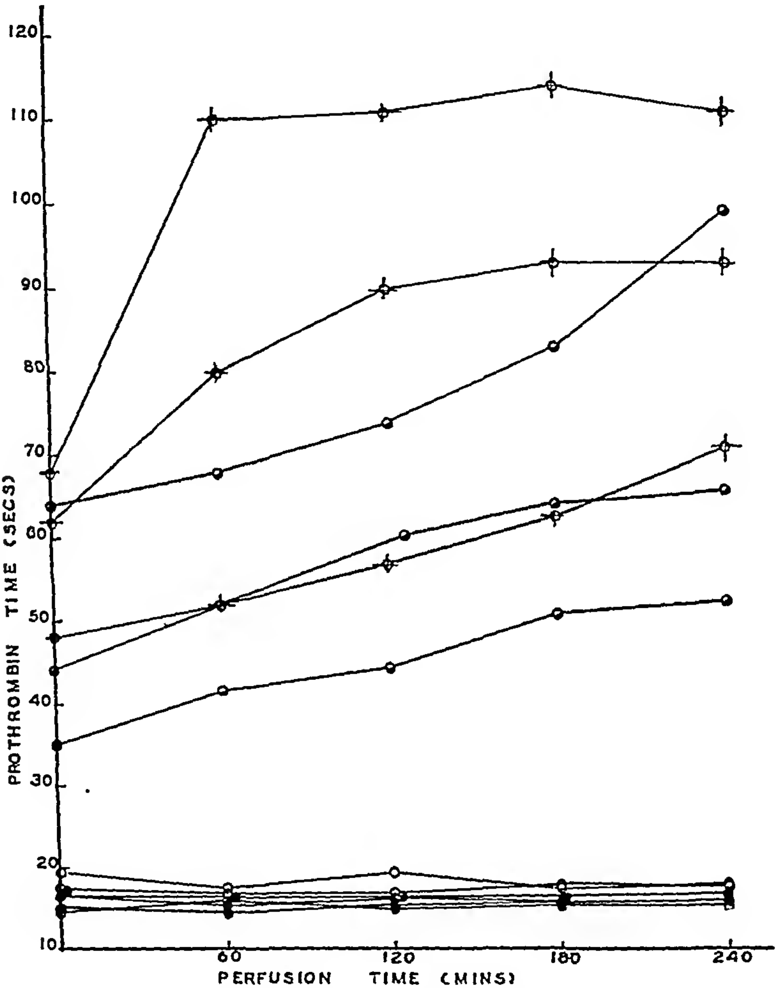

FIG. 1. PERFUSION OF POOLED RAT BLOOD

a. O, normal blood through the liver of a normal rat. b. ●, normal blood through the liver of a dicumarol prepared rat. c. ⌧, blood from dicumarol prepared rats through the liver of a dicumarol prepared rat. d. ◐, blood from dicumarol prepared rats through an open cannula.

prepared liver in the system is not greater than when no liver is present. It seems justified to conclude that there is no destructive activity in the dicumarol

prepared liver under the perfusion conditions. Further, the perfusion of normal blood through the liver of a dicumarol treated rat resulted in no decrease in the

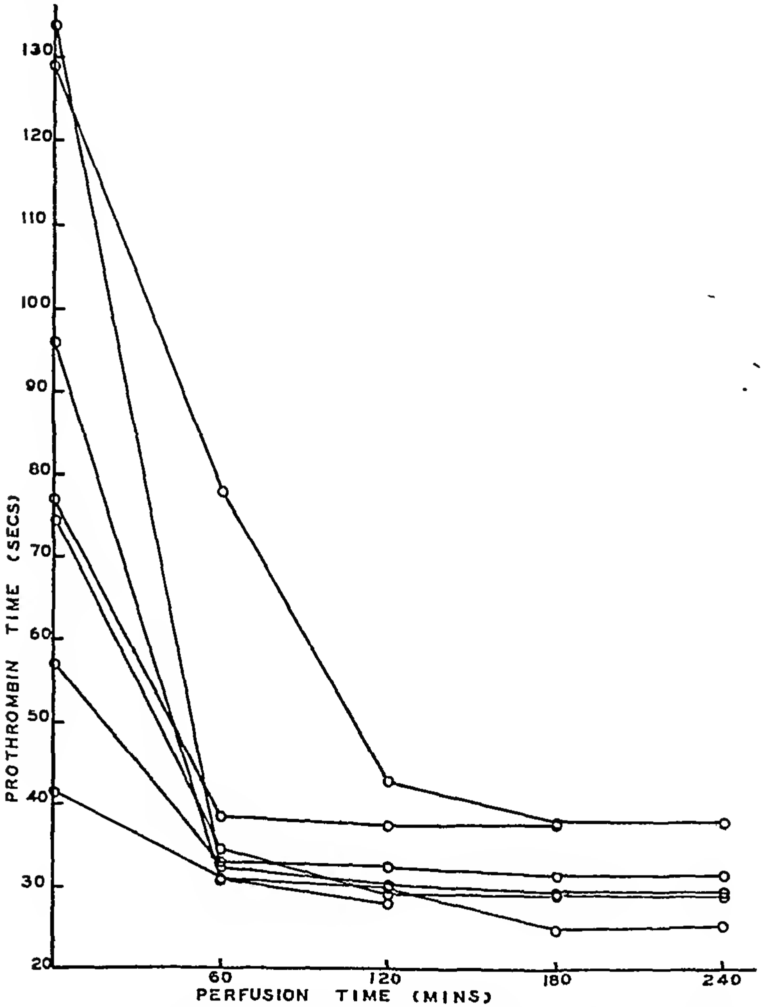

FIG. 2. PERFUSION OF BLOOD FROM DICUMAROL TREATED RATS THROUGH THE LIVER OF A NORMAL RAT

prothrombin activity. It seems highly unlikely that dicumarol causes increased destruction of prothrombin by the liver.

In contrast to the lack of destructive activity of dicumarol, livers from normal

rats were able to increase the prothrombin activity of blood deficient in prothrombin due to the action of dicumarol. If the results of these data are compared with the hyperbolic curve made by plotting prothrombin time against percentage of diluted pooled rat blood (2), it is found that there is an increase of from 4 to 12 per cent in prothrombin. While the increase does not approach normal levels, nevertheless an increase of about 10 per cent in the prothrombin level of the perfusate is significant. The volume of the perfusate was always from 50 to 60 ml., which is approximately twice the blood volume of the rats used as liver donors. Although these values are certainly not quanitative, they do represent only a clear-cut increase in the prothrombin activity of perfused blood.

TABLE 3

Perfusion of pooled whole blood through the liver from a dicumarol rat

The prothrombin times are recorded in seconds (average of three determinations)

	PERFUSION TIME IN MINUTES					PROTHROMBIN TIME OF LIVER DONATING RAT
	0	60	120	180	240	
Blood from normal rats						
#1	16.5	15.5	16.5	16.0	—	26.5
#2	15.0	14.5	15.5	15.5	16.0	36.0
#3	17.5	17.5	17.5	17.5	17.0	450.0
Blood from dicumarol treated rats						
#1	62.5	80.0	90.0	93.0	93.0	55.0
#2	48.0	52.0	57.0	63.0	71.0	65.0
#3	68.0	110.0	111.0	114.0	111.0	105.0

SUMMARY AND CONCLUSIONS

Pooled blood from white rats was perfused through the isolated liver of a rat in an apparatus so arranged as to permit the reperfusion of from 50 to 60 cc. over a period of three to four hours, under standardized conditions.

Normal blood perfused through the liver of a normal rat resulted in no change in the prothrombin activity.

Blood from dicumarol treated rats perfused through an open cannula decreased slightly in prothrombin activity. Similar blood perfused through the liver of a dicumarol treated rat, decreased in prothrombin activity to the same extent as when no liver was in the circuit.

Perfusion of normal blood through livers of dicumarol treated rats resulted in no change in the prothrombin levels.

Blood from dicumarol treated rats perfused through livers of normal rats increased significantly in prothrombin activity.

It may be concluded that under the conditions of perfusion of an isolated organ, livers from dicumarol treated animals do not cause a destruction of prothrombin. Secondly, the normal isolated liver of the rat is capable of increasing

the prothrombin activity of blood deficient in prothrombin activity as a result of treatment with dicumarol. This is further evidence of the liver being the site of formation of prothrombin, and that dicumarol decreases the capacity of the liver to produce prothrombin.

REFERENCES

(1) Warren, R., and Rhoads, J. E.: Am. J. M. Sc., 198: 193, 1939.
(2) Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Am. J. Physiol., 114: 667, 1936.
(3) Smith, H. P., Warner, E. D., and Brinkhous, K. M.: J. Exper. Med., 66: 801, 1937.
(4) Quick, A. J.: J. A. M. A., 110: 1658, 1938.
(5) Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P.: J. Biol. Chem., 138: 1, 1941.
(6) Jaques, L. B.: Biochem. J., 37: 344, 1943.
(7) Mellanby, J.: Proc. Roy. Soc. London sB 107: 271, 1930.
(8) Kazal, L. A., and Arnow, L. E.: Arch. Biochem., 1: 169, 1942.
(9) Quick, A. J.: The Hemorrhagic Diseases. 1942, Chas. Thomas, Springfield, Ill.

CARDIOVASCULAR EFFECTS OF SODIUM CAPRYLATE IN THE CAT*

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Received for publication December 19, 1946

The sodium salts of caprylic acid and of other lower fatty acids, when injected intravenously, cause ventricular ectopic rhythms similar to those produced by toxic doses of digitalis (1). Sodium caprylate increases the force of contraction of the perfused isolated frog heart and decreases the degree of dilatation (2). This effect is more pronounced in hearts which are failing than in fresh hearts, an action also similar to that produced by digitalis. The experiments to be reported here were designed to determine whether or not doses of sodium caprylate lower than those which produced cardiac arrhythmias had a digitalis-like effect on the mammalian cardiovascular system.

METHODS. The cats were anesthetized with sodium pentobarbital, using an initial intraperitoneal dose of 37.5 mgm. per kgm. and maintaining anesthesia with additional small intravenous doses as needed. Under artificial respiration by rhythmic positive pressure through a tracheal cannula the chest was opened and the heart freed from the pericardium and placed in a cardiometer up to the coronary sulcus. Ventricular volume changes were recorded on a kymograph by air transmission activating a writing lever on a loose rubber diaphragm over a tambour of 5 cm. diameter. The system was calibrated on each animal with the heart in the cardiometer so that the approximate "stroke volume" could be read from the record. (The volume which will be referred to as stroke volume in this paper was actually the systolic change in external volume of both ventricles.) Arterial pressure was recorded from a carotid artery by a mercury manometer. Venous pressure was measured in an external jugular vein at suitable intervals by the venous inflow method, using a heparinized Ringers solution in the manometer system. Injections were made through cannulae in the femoral or jugular veins. The heart rates were counted from the records and "cardiac outputs" were calculated using the measurement of "stroke volume" as explained above.

The sodium caprylate was made up in aqueous solutions of 0.025M to 1.5M concentration, adjusted to pH 7.4. The doses varied between 0.075 and 0.75 mM per kgm.

RESULTS AND DISCUSSION. *The Effect of Varying Doses of Sodium Caprylate.*—The intravenous injection of 0.075, 0.225, 0.45, and 0.75 mM per kgm. (3 cc. per kgm. of 0.025M, 0.075M, 0.015M, or 0.25M) of sodium caprylate produces cardiovascular effects similar in the different doses but of progressively increasing intensity. These effects are an increase in diastolic volume with a less marked increase in systolic volume and a consequent increase in "stroke volume", a rise in venous pressure, a moderate slowing of the heart rate, an increased cardiac output, and a slight rise in arterial pressure followed by a much greater fall and later by a gradual rise often to above the original level. These changes are produced in part by the sodium caprylate itself and in part by the volume of fluid injected. The latter factor, which has been evaluated in our

* This study was aided by a grant from the Fluid Research Fund of the Stanford University School of Medicine.

experiments by the injection of the same volume (3 cc. per Kgm.) of Ringers solution, includes only the results to be expected from an increase in blood volume and consequently in cardiac filling.

The effects of caprylate administration began immediately but their duration varied so that the maximum change in the different measurements of circulatory activity occurred at different times following the injection. All the effects subsided in 10 to 20 minutes. A typical example is shown in figure 1.

Because of the variation in the time course of these cardiovascular changes, a time had to be arbitrarily selected at which to make a quantitative comparison

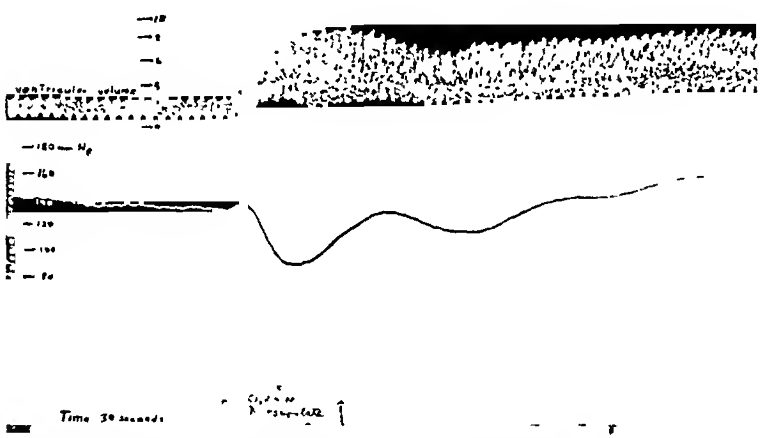

FIG. 1. The effect of 0.75 mM per kgm. of sodium caprylate on the ventricular volume and arterial pressure of a cat. The upstroke indicates diastole, the downstroke systole; the difference between diastolic and systolic volume is in cc. The injection was given between the arrows.

of the effect of various doses of caprylate. Since the increase in stroke volume was the most regular feature of the changes observed, the moment when it reached its maximum was chosen as the time for all readings, and therefore the other readings do not necessarily represent maximal changes.

The various doses mentioned above and an equal volume (3 cc. per kgm.) of Ringers solution were given to each cat in sequence with a suitable waiting period for recovery between injections. In successive animals the first dose in the sequence was changed in rotation, with the other injections following in order.

The results of this series of experiments are shown in table 1 and figure 2. They are stated as the mean of the individual per cent changes in experiments on 2 to 7 animals. The stroke volume is increased with increasing doses and

is in all cases greater than that with the same volume of Ringers solution. The heart rate was decreased with increasing doses but not enough to offset the increase in stroke volume so that the cardiac output also increased with increasing

TABLE 1

A comparison of the effect of Ringers solution and varying doses of sodium caprylate on the cardiovascular system in the cat

The volume per kgm. was the same in all cases. The figures are the mean values for the number of experiments indicated; the per cent change is the mean of the individual per cent changes and not the per cent change of the mean difference given in the table. The figures for diastolic and systolic volume are arbitrary scale readings. "Stroke volume" refers to the change in ventricular volume per beat as recorded by the cardiometer (see "Methods"). "Cardiac output" is the "stroke volume" multiplied by the heart rate.

DOSE mM/KGM.		VENOUS PR. MM. OF WATER	ARTERIAL PR. MM. OF MERCURY	DIAST. VOL.	SYST. VOL.	"STROKE VOLUME" MLS.	"CARDIAC OUTPUT" MLS. PER MIN.	HEART RATE BEATS PER MIN.
Ringers sol.	No. cats	5	7	7	7	7	7	7
	before	95	116	5.0	2.8	2.2	372	172
	after	103	115	6.4	3.3	3.1	498	166
	difference	+8	−1	+1.4	+0.5	+0.9	+126	−6
	% change	+9.6	−1.8			+43.2	+39.3	−2.9
0.075	No. cats	3	6	6	6	6	6	6
	before	115	116	5.8	4.0	2.0	339	169
	after	129	113	6.9	4.1	2.9	429	154
	difference	+14	−3	+1.1	+0.1	+0.9	+90	−15
	% change	+10.0	−3.3			+54.6	+43.4	−8.3
0.225	No. cats	2	4	4	4	4	4	4
	before	95	110	3.6	1.8	1.8	291	159
	after	104	114	5.5	2.9	2.5	379	152
	difference	+9	+4	+1.9	+1.1	+0.7	+88	−7
	% change	+12.1	+1.2			+65.4	+59.1	−4.3
0.45	No. cats	2	5	5	5	5	5	5
	before	74	117	4.8	2.1	2.7	445	177
	after	90	91	7.1	3.3	3.8	518	151
	difference	+16	−26	+2.3	+1.2	+1.1	+73	−26
	% change	+19.3	−23.0			+72.3	+44.0	−14.9
0.75	No. cats	3	6	6	6	6	6	6
	before	105	115	5.2	2.8	2.4	369	163
	after	121	89	7.7	3.9	3.8	502	134
	difference	+16	−26	+2.5	+1.1	+1.4	+133	−29
	% change	+16.0	−24.5			+89.2	+56.6	−17.4

doses. The venous pressure in general increased with the dose and was regularly greater than the increase due to the volume of fluid injected. The arterial pressure was lowered to an increasing extent with increasing doses. The fact that those changes do not in all cases represent maximal changes, but only the

per cent change when the stroke volume increase was maximal, may account for the failure of the values, other than those for stroke volume, to change progressively and uniformly with increasing dose.

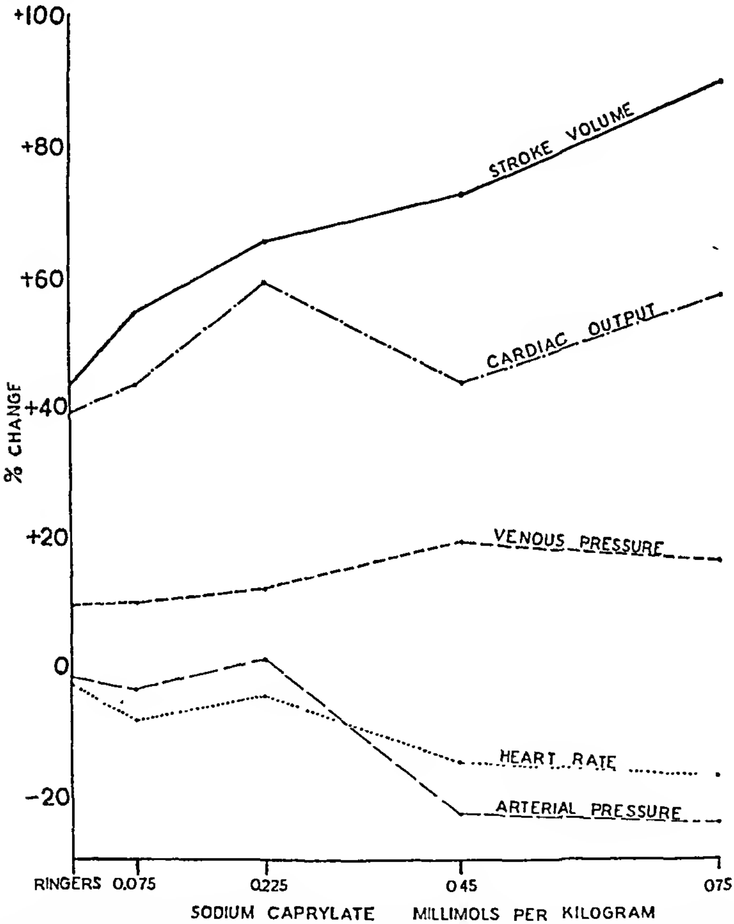

FIG. 2. A comparison of the effect of Ringers solution and varying doses of sodium caprylate on the per cent change in stroke volume, cardiac output, venous pressure, heart rate and arterial pressure in the cat. The volume of fluid injected per kgm. was the same in all cases.

The tremendous increases in stroke volume were produced by a sudden marked dilatation of the heart with less increase in systolic volume than in diastolic volume. The cardiac output was also increased even though the rate was decreased slightly. [illegible] increase in stroke volume and cardiac output was

accompanied by an increase in diastolic volume and venous pressure, it was at least apparently not a digitalis-like action as had been suggested by the work on the isolated frog heart (2).

The fall in arterial pressure during the period of increased cardiac output demonstrated that the caprylate was causing a decrease in peripheral resistance, possibly by a direct action on smooth muscle in the peripheral vessels. Such a peripheral vasodilatation could account in part at least for the rise in venous pressure on the postulation of an increased venous return from the capillaries which would in turn cause an increase in cardiac filling, leading to the cardiac dilatation and increased stroke volume. The venous pressure might also be raised in part by a venoconstrictor action.

The effects described were produced when the animals were in good condition. After several hours of artificial respiration and a series of experimental procedures, the hearts dilated with a decreased stroke volume, a drop in arterial pressure, and an increase in venous pressure. When a cat was in such a condition, even the smaller doses of caprylate not only did not improve the circulation but rapidly made it worse. The heart dilated still further and its systolic ejection was decreased to very low levels. The heart rate decreased markedly and extrasystoles and other arrhythmias often appeared. The arterial pressure quickly dropped almost to zero. Complete stoppage of the heart usually occurred, under such conditions, within 5 minutes after the caprylate was injected. This lethal effect was the same regardless of the conditions under which the preliminary cardiac failure was produced, i.e., whether it followed improper adjustment of the artificial respiration, repeated doses of caprylate, or injection of very large quantities of Ringers solution. A comparison was made with Lanatoside C (kindly furnished by the Sandoz Chemical Works, Inc.) in a dose of 0.1 mgm. per kgm. The latter caused dilated hearts to increase their stroke volume while decreasing their diastolic volume. This increase in the effectiveness of the heart was reflected in a rising arterial pressure and a decreasing venous pressure, results quite unlike those obtained with the caprylate.

The Effect of Caprylate on Stroke Volume at a Given Diastolic Volume.—It was thought that the peripheral effects of the sodium caprylate might be masking a possible digitalis-like action on the heart, an action that was manifest in the experiments on the frog hearts because they were working under conditions of constant venous pressure and peripheral resistance. Therefore a second series of experiments was carried out to determine the effect of caprylate under conditions of controlled diastolic volume.

First a dose of 0.75 mM per kgm. of 1.5M sodium caprylate was given, the more concentrated solution being used to minimize the effect of the volume of fluid injected. The maximum diastolic volume was recorded and the animal allowed to recover. Then Ringers solution was forced rapidly into the vein until the diastolic volume reached the maximum attained during the response to caprylate. Pairs of measurements were made of stroke volumes with caprylate and with Ringers when diastolic volumes were equal. Twenty-six pairs of data were taken from 11 cats. They show a tendency for the stroke volume

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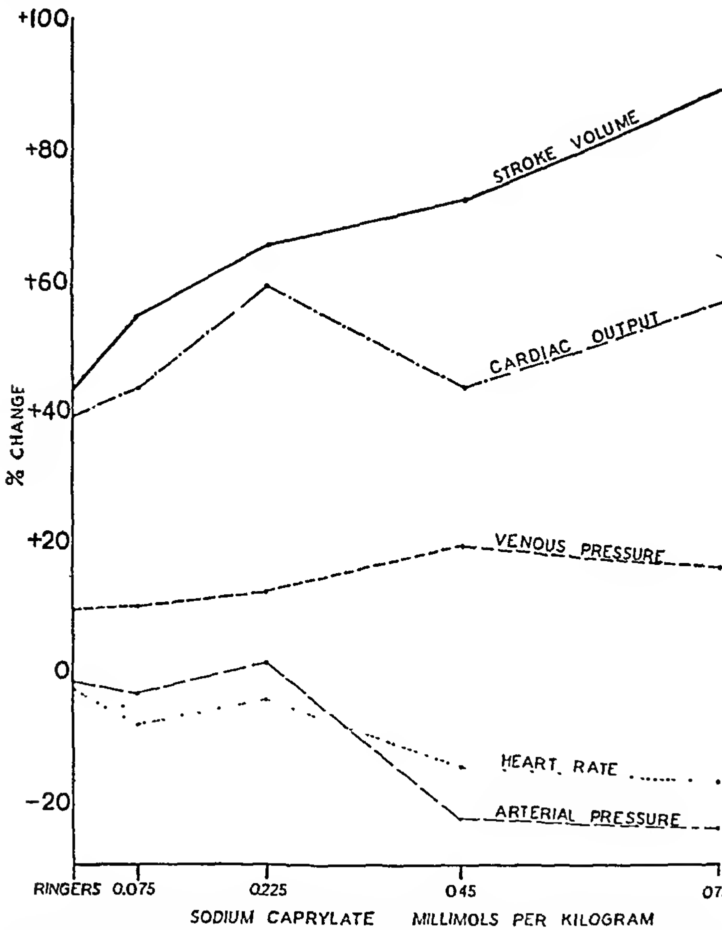

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to be greater when the cardiac dilatation was induced by caprylate than when it was induced by Ringers solution, the means being 4.12 and 3.85 mls. respectively. The arterial pressures against which the hearts were discharging these stroke volumes averaged 124 mm. Hg after caprylate as compared with only 99 mm. Hg after Ringers solution. Accordingly, the work done by the heart per beat, roughly estimated by the product of the stroke volume and the arterial pressure, is approximately one-third greater when a given diastolic volume is evoked by caprylate than when the same diastolic volume is evoked by saline injection. Thus there is some evidence that caprylate has a digitalis-like effect on the mammalian heart similar to that on the frog heart although usually such cardiac effects are obscured by a greater effect on other parts of the circulatory system.

The Effect of Caprylate on Diastolic Volume at a Given Venous Pressure.—Since various doses of sodium caprylate increased the diastolic volume more than the injection of an equal volume of Ringers solution, the question of the cause of this cardiac dilatation arose. The possibility that the caprylate increased the distensibility of the heart muscle directly was explored in a third series of experiments. Again 0.75 mM per kgm. of 1.5M caprylate was given and the animal allowed to recover. Then Ringers solution was force rapidly into the femoral vein until the venous pressure at the external jugular equalled the maximum venous pressure obtained under the influence of caprylate. Pairs of measurements were made of diastolic volume under caprylate and under Ringers solution when the venous pressures were equal. The heart rates were also approximately equal (averaging 166 with caprylate and 170 with Ringers solution). Fourteen such pairs of data were obtained from 5 cats, the mean scale readings for diastolic volume (this is not an actual volume) being 8.17 and 7. 70 respectively. Since the systolic volumes differed in the same direction and to approximately the same extent (being 4.91 units with caprylate and 4.40 with Ringers solution), the small difference in diastolic volumes cannot be construed as evidence for a greater diastolic distensibility of the ventricular muscle under the influence of caprylate. It seems then that the changes in diastolic volume produced by sodium caprylate are primarily a reflection of the peripheral effects on venous return and venous pressure, the mechanism of which is not known.

CONCLUSIONS

These experiments show that, while sodium caprylate has an action resembling that of digitalis on the isolated frog heart, and possibly on the cat heart in the latter this effect is concealed by a greater action on peripheral vessels. This latter effect may be a dilatation of the arterioles, an opening of arteriovenous connections or venoconstriction, or some combination of these; in any event, it results in decreased arterial pressure and increased venous pressure which in turn causes increased diastolic and stroke volumes, and a rise in cardiac output. The effect on the peripheral vessels could be either a direct action on their smooth muscle or one mediated through nervous mechanisms.

Furthermore, when caprylate is given to a cat whose heart is already dilated

and weak, it causes rapid death due to further cardiac dilatation with failing output. This is in sharp contrast to the greater effect which digitalis preparations have on failing hearts. Accordingly, such digitalis-like effect as caprylate may have in mammalian hearts is not such as to deserve consideration as a therapeutic agent in cardiac failure.

The increase in venous pressure is of considerable interest. Vasodilator substances, such as the nitrites, commonly cause a fall in venous pressure and cardiac output. Caprylate, however, while clearly a vasodilator agent, causes a rise in venous pressure and an increase in cardiac output. It may prove of value to have a substance with this unusual combination of effects for use in the analysis of various circulatory states.

SUMMARY

1. Sodium caprylate injected intravenously into cats in doses of 0.075 to 0.75 mM per kgm. causes an immediate temporary dilatation of the heart, increased stroke volume, cardiac output and venous pressure, and a decreased heart rate and arterial pressure.

2. The effects are qualitatively the same with varying doses and vary in a roughly quantitative way with the dose.

3. There is a small increase in stroke volume with caprylate over that shown when the diastolic volume is increased to an equal degree by injection of Ringers solution. Since the arterial pressure with caprylate is also greater than with Ringers solution, the work done by the heart per beat at constant diastolic volume is greater with caprylate than with Ringers solution. Accordingly, caprylate may be said to show a digitalis-like action on the cat heart.

4. Since there is no detectable increase in the diastolic distensibility of the ventricular muscle, nor any impairment of the force of systolic contraction, the dilatation of the heart is to be attributed to the increase in venous return.

5. It is suggested that sodium caprylate has a peripheral vasodilator action which masks its digitalis-like effect on the heart. This is one possible cause of the rise in venous pressure.

BIBLIOGRAPHY

(1) Hall, V. E., and Waldman, M. S., J. Pharm. Exper. Therap., 88, 221, 1946.
(2) Elliott, H. W., Kipple, H. M., and Hall, V. E., J. Pharm. Exper. Therap., 89, 14, 1947.

ON THE MECHANISM OF PARALYSIS RESULTING FROM TOXIN OF CLOSTRIDIUM BOTULINUM

The Action of the Toxin on Acetylcholine Synthesis and on Striated Muscle*

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Received for publication December 30, 1946

The toxin of *Clostridium botulinum* causes diplopia, blepharoptosis, loss of accomodation, dilatation of pupils, facial weakness, decreased salivation, respiratory weakness, difficulty in swallowing, chewing and talking, as well as generalized and often extreme weakness and fatigability (1–8). The effect of the toxin of *Cl. botulinum* has been said to be curare-like (5–7, 9). The term "curare-like effect" is usually used to describe a dysfunction at the myoneural junction, manifesting itself in decreased ability of the muscle to respond to indirect stimulation and unaltered ability of the muscle to respond to direct stimulation. However, an apparent curare effect may result from involvement of different mechanisms: 1) by prevention of the action of acetylcholine on striated muscle (as does curare) and 2) by inhibition of acetylcholine synthesis (10).

In the following an attempt was made to ascertain whether toxins of *Cl. botulinum* modify either or both of these processes.

A. Effect on Muscle Contraction Induced by Acetylcholine. The effects of the toxins and of d-tubocurarine on the response of striated muscle to acetylcholine were investigated following a method described previously (11). Shortening of the rectus abdominis muscle was induced by immersion in a frog Ringer's solution containing acetylcholine bromide (50 μg per 100 cc.) for 2 minutes. (In two minutes the muscle has reached almost maximum contraction.) The shortening of the muscle was registered by an isotonic lever on a kymograph. After stabilization, between two shortenings induced by the acetylcholine solution, instead of washing with Ringer's solution for 10 minutes, the muscle was washed for 5 minutes and was immersed for 5 minutes in Ringer's solution containing one of the substances to be investigated (pH 7). The substances did not induce muscle contraction.

Control. Muscles were immersed only in Ringer's solution and shortening was induced with the acetylcholine solution as described above. The repeatedly induced shortenings, each lasting two minutes, were of the same magnitude for at least three hours. This period of time was longer than the duration of the experiments described.

* This study was aided by a grant from the John and Mary R. Markle Foundation.

Calculation. The amount of shortening of muscle after immersion in the solution of the compounds was expressed as percentage of the amount of shortening of the same muscle before immersion. All results deviating from 100 per cent by more than twice the square root of the sum of the squares of the standard error of the controls and the standard error of the experiments were considered significant

$$2\sqrt{\text{S.E.}^2(\text{control}) + \text{S.E.}^2(\text{experiment})}$$

The S.E.(control) was ± 1.3.

Results. The rectus abdominis muscle was immersed in solutions containing the toxin of *Cl. botulinum* (Type A and B) in concentrations from 1 to 100 LD_{50}[1] and 10,000 LD_{50} per 100 cc. of Ringer's solution. The response of the muscle to acetylcholine was not modified by concentrations from 1 to 100 LD_{50}. The response of muscle to acetylcholine increased after immersion in solutions containing 10,000 LD_{50} toxin per 100 cc. of Ringer's solution. The shortening was 122 ± 2.0 per cent (average of 10 experiments) after immersion in a solution of toxin Type B(No. – 199) and was 131 ± 3.0 per cent (average of 10 experiments) after immersion in a solution of toxin Type A(No.A-213 #3).

On the other hand, the response of the muscle to acetylcholine decreased after immersion in solutions of d-tubocurarine. The shortening was 82 ± 1.1 per cent after immersion in a solution containing 0.01 mgm. of d-tubocurarine per 100 cc.; 49 ± 0.9 per cent after immersion in a solution of 0.1 mgm. per 100 cc.; and 8 ± 0.5 per cent after immersion in a solution of 1 mgm. per 100 cc. (average of 10 experiments for each group).

B. Effect of Toxins on Acetylcholine Synthesis. I. *Frog Brain.* a) *Aerobic Experiments.* The effects of toxins on the synthesis of acetylcholine were investigated using a modified method of Quastel, Tennenbaum, and Wheatley (12, 13). Mixtures containing varying amounts of the toxins (pH adjusted to 7.4), minced fresh frog brain (100 mgm.), physostigmine salicylate (3 mgm.), and frog-Ringer's solution (3 cc.) were shaken and were incubated aerobically for 4 hours at 37° C. After incubation, the amount of acetylcholine synthesized was assayed biologically on the sensitized rectus abdominis muscle of the frog.

Control. Incubated mixtures containing frog brain, physostigmine, Ringer's solution, and boiled toxin in varying amounts served as controls.

Calculation. The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of incubated mixtures, the acetylcholine content of identical non-incubated mixtures. The amount of acetylcholine synthesized in the mixtures containing boiled toxin was taken as 100 per cent. The acetylcholine content of the mixtures containing the various toxins used was expressed as per cent of the control.

Results. The results are given in Table 1. The synthesis of acetylcholine decreased in the presence of small amounts of the toxins. A 20 per cent decrease was found in the presence of 0.1 LD_{50} (mouse) toxin per 100 mgm. of frog brain.

Minced brain contains a large number of intact cells. The enzyme that syn-

[1] LD_{50} (mouse) is the amount of toxin that killed 50 per cent of mice (12 groups of 4 mice each) within 4 days after an intraperitoneal injection. The factors (0.1–10,000) express multiples of the amount of LD_{50}.

thesizes acetylcholine is intracellular. To achieve a better disruption of the brain cell structure a homogenate of frog brain was prepared by the apparatus of Potter and Elvehjem (14). The toxin was incubated with the homogenate and the synthesis of acetylcholine was ascertained as described above. The inhibition of acetylcholine synthesis in the homogenized brain was greater than in the mixtures containing minced brain (Table 1).

b) *Anaerobic Experiments.* A series of experiments was also performed under anaerobic conditions since there is some evidence that the enzyme utilizes different precursor substances under aerobic conditions than under anaerobic conditions (15). The results are given in Table 1. The percentage decrease in the synthesis of acetylcholine in these anaerobic experiments was similar to that in the previously described aerobic experiments.

TABLE 1

Effect of toxins on acetylcholine synthesis

TOXINS	AMOUNT OF ACETYLCHOLINE SYNTHESIZED (PER CENT OF CONTROL; EACH NUMBER REPRESENTS THE AVERAGE OF TEN SEPARATE EXPERIMENTS) Amount of toxin (LD_{50} mouse) added to 100 mgm. frog brain:			
	100	10	1	0.1
Aerobic Incubation				
Minced brain				
Type A (No. A-213 #3)	35 ±2.9	49 ±2.6	73 ±2.1	80 ±1.5
Type B (No. B-195 #3)		39 ±1.3	70 ±1.4	79 ±1.2
Type B (No. B-199)	30 ±4.0	44 ±2.2	60 ±3.4	77 ±2.0
Homogenized brain				
Type A (No. A-213 #3)	24 ±3.3			
Type B (No. B-199)	19 ±4.2			
Anaerobic Incubation				
Minced brain				
Type A (No. A-213 #3)	25 ±3.2	33 ±3.9	58 ±3.5	
Type B (No. B-195 #3)	21 ±2.8	30 ±2.7	50 ±2.8	

II. *Mouse Brain.* The effect of the toxin of *Cl. botulinum* Type B on the synthesis of acetylcholine by mouse brain was investigated to ascertain whether this toxin also modifies the activity of the enzyme obtained from brain of warm-blooded animals. The experiments were performed as described above, except that one-half of the minced mouse brain was used as the control and the other half as the experimental specimen (division occurred after mincing, by weight). The brain was suspended in Locke's solution instead of Ringer's solution and was incubated aerobically.

In the presence of 12 LD_{50} of toxin of *Cl. botulinum* the synthesis of acetylcholine was markedly reduced; with Type B (Batch No. B-195 #3) to 54 ± 2.7 per cent (average of 10 mice), and (Batch No. B-199) to 50 ± 3.0 per cent (average of 10 mice), of the control value.

III. *Effect of Toxin Injected Into Mice on the Synthesis of Acetylcholine.* Mice

were injected with 1 LD_{50} dose of the toxin. The mice were killed by severing the head from the body the third day after subcutaneous injection of the toxin. The brain was removed, weighed, and minced. Half of the brain was suspended in Locke's solution (3 cc.) and physostigmine salicylate (3 mgm.) was added. The amount of acetylcholine synthesized by brain of non-injected mice taken from the same batch of animals served as control.

Brain obtained from the injected animals synthesized less acetylcholine (60 $\pm$ 3.3 per cent (average of 15 mice)) than brain of non-injected animals.

DISCUSSION

The toxin of *Cl. botulinum* is one of the most potent agents known to decrease acetylcholine synthesis. One MLD (mouse) of purified Type A toxin contains only 8.3×10^{-9} mgm. nitrogen (16). There is some evidence that the enzyme for the synthesis of acetylcholine utilizes different precursor substances under aerobic conditions as compared with anaerobic conditions (15). The similarity in the amounts of decrease of acetylcholine synthesis under both aerobic and anaerobic conditions suggests that the toxin affects the enzyme and not the substrate.

Small amounts of the toxins were sufficient to decrease the synthesis of acetylcholine in both *in vivo* and *in vitro* experiments. The inhibition *in vivo* was not complete, probably because the cell components protect the intracellular enzyme from contact with the toxin. Such incomplete inhibition of acetylcholine synthesis may explain why animals so poisoned show responses of the effector apparatus when the nerve is stimulated.

The effect of the toxin of *Cl. botulinum* has been considered to be like that of curare (5–7, 9). The above experiments indicate that the defect is more like that induced by agents that interfere with acetylcholine synthesis. Substances that decrease acetylcholine synthesis cause functional defects at the myoneural junction (10), though these substances, in contrast to curare, usually do not decrease the response of striated muscle to acetylcholine. The above experiments make it appear likely that the toxin of *Cl. botulinum* in causing paralysis acts mainly by decreasing acetylcholine synthesis.

SUMMARY

1. The effects of toxins of *Cl. botulinum*, Types A and B, on the synthesis of acetylcholine and on the responsiveness of striated muscle to acetylcholine were investigated.

2. The synthesis of acetylcholine decreased in the presence of the toxins.

3. The responsiveness of striated muscle to acetylcholine was not modified by low concentrations of the toxins (from 1 to 100 LD_{50} per 100 cc. Ringer's solution) and was moderately increased by high concentrations of the toxins (10,000 LD_{50} per 100 cc.).

4. It is suggested that decreased acetylcholine synthesis, as far as it is relevant to myoneural function, is mainly responsible for the "functional paralysis" observed in animals affected by the toxins of *Cl. botulinum*.

The authors wish to express their gratitude to Dr. A. Gilman for suggesting the use of toxin of *Cl. botulinum* and to Lederle Laboratories for the generous supply of the toxin.

BIBLIOGRAPHY

1. Fischer, Zschr. Klin. Med., 59, 58, 1916
2. Dickson, E. C., Monograph, Rockefeller Institute Med. Research, July, 1918
3. Dickson, E. C., and Shevky, R., Proc. Soc. Exp. Biol. and Med., 18, 313, 1922
4. Dickson, E. C., and Shevky, R., J. Exper. Med., 38, 327, 1923
5. Edmunds, C. W., and Long, P. L., J. A. M. A., 81, 542, 1923
6. Schuebel, K., Archiv exp. Pathol. Pharmacol., 96, 193, 1923
7. Edmunds, C. W., and Kieper, G. E. Jr., J. A. M. A., 83, 495, 1924
8. Huertle, R., and Gross, H., Dt. Med. Wschr., 60, 1232, 1934
9. Schuebel, K., Dt. Med. Wschr., 47, 1047, 1921
10. Torda, C., and Wolff, H. G., Amer. J. Physiol., 147, 384, 1946
11. Torda, C., and Wolff, H. G., Amer. J. Physiol., 145, 419, 608, 1946
12. Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., Biochem. J., 30, 1668, 1936
13. Torda, C., and Wolff, H. G., J. Clin. Invest., 23, 649, 1946
14. Potter, V. R., and Elvehjem, C. A., J. Biol. Chem., 114, 495, 1936
15. Lipton, M. A., and Barron, E. S. G., J. Biol. Chem., 166, 367, 1946
16. Lamanna, C., McElroy, O. E., and Eklund, H. W., Science, 103, 613, 1946

STUDIES ON VERATRUM ALKALOIDS

VII. Receptor Areas in the Coronary Arteries and Elsewhere as Revealed by the Use of Veratridine[1]

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Received for publication December 31, 1946

The veratrum alkaloids, apart from their intrinsic interest as substances with particular pharmacological properties, are beginning to become useful in the analysis of physiological responses in the mammalian organism. While the "veratrinic responses" of nerve and muscle have been familiar objects of investigation, the cardiovascular and respiratory actions of these alkaloids have not been studied so intensively. In 1867, von Bezold and Hirt (1) observed that the fall of blood pressure and heart rate which followed the intravenous injection of veratrine was abolished by cutting the vagi. They considered this action to be of reflex nature. On the basis of experiments using an innervated dog heart-lung preparation with separate perfusion of the head, Krayer, Wood and Montes (2) concluded that this reflex originates predominantly in the viscera of the chest. According to Jarisch and Richter (3), and Richter and Amann (4), the most important part of the impulses for the vasodepressor reflex come from the myocardium of the ventricles, and only a minor proportion from the lungs. These conclusions were based on experiments in cats in which veratrine was injected before and after interruption of the afferent cardiac branches of the vagus, by section or by local anesthesia; and on experiments in which veratrine was injected into the left pulmonary artery during occlusion of the circulation through the left lung. Krayer and Acheson (5) in their review of the pharmacology of the veratrum alkaloids emphasized the caution required in the interpretation of these results because of the difficulty of separating experimentally receptor areas in the atria and ventricles from those already recognized in and around the great vessels. The work of Amann and Schaefer (6), which was not known to Krayer and Acheson when writing their review, shows that various afferent fibres can be found in the cardiac branches of the vagus, which carry bursts of electrical activity in phase with the heart beat, and which can be caused to fire off continuously by the injection of veratrine.

In this paper a somewhat different experimental approach has been used, viz., injection of small quantities of the veratrum alkaloids into the vascular supply of the heart and lungs in order to localize the receptor areas more closely. The results obtained confirm the conclusion that the heart is the principal site of action and suggest that the afferent impulses arise predominantly from the left ventricle.

[1] The expenses for animals used in this study were defrayed from grants received from the Rockefeller Foundation and from Irwin, Neisler and Company, Decatur, Illinois.

[2] Rockefeller Travelling Fellow.

METHODS. *Perfusion of the main coronary arteries.* This preparation was devised in order to facilitate the injection of drugs into the coronary arteries. It is evidently similar to that referred to by Gregg and Shipley (7) in dogs. In dogs the whole left coronary artery and in cats both left and right coronary arteries were perfused. The animal was anesthetized with chloralose (0.08 mg./kgm.) or pentobarbital[3] (25 mg./kgm. intravenously, 35 mg./kgm. intraperitoneally) and the chest opened under artificial respiration. A

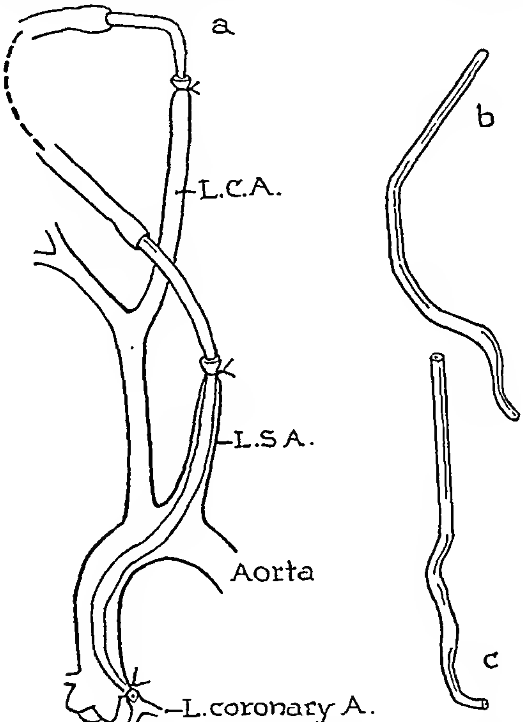


FIG. 1. Diagram of preparation for injecting substances into the left coronary artery in the cat. The left carotid artery (L. C. A.) is connected by a cannula (a) and a flexible rubber tube to a glass tube passed down the left subclavian artery (L. S. A.) and tied into the mouth of the left coronary artery. The glass tube used is shown in lateral (b) and antero-posterior (c) views, (reduced to ¾ of natural size.)

ligature was passed around the origin of the coronary artery to be perfused. In cats this was usually simple, though sometimes the left coronary artery had a multiple origin from the aorta which made the preparation impossible. In dogs it was much more difficult on the left side, because of the high origin of the septal artery at the bifurcation into left circumflex and anterior descending branches. A short length of the left subclavian artery was prepared, the animal was given heparin[4] 3–5 mg./kgm. intravenously, and a cannula

[3] The Sodium pentobarbital was generously supplied by Abbott Laboratories, North Chicago, Illinois.

[4] The heparin used in this study was supplied by Eli Lilly and Company, Indianapolis, Indiana through the kindness of Dr. K. K. Chen.

tied into one carotid artery. The latter was connected by a U-piece and a short length of flexible rubber tubing to a glass tube shaped to fit the curves of the left subclavian artery and ascending aorta. This tube was pushed gently down the subclavian artery and guided by touch into the mouth of the coronary artery, where it was tied. The circulation through the coronary artery was not interrupted during this procedure, and the blood pressure and electrocardiogram were unaltered, provided that the tube was a good fit. Any drug injected into the flexible rubber tubing was then carried directly into the coronary artery (fig. 1). The left subclavian artery was selected for this purpose because it is so much larger than the carotids in the cat; a proportionately larger glass tube could therefore be used, offering less resistance to the flow of blood. Equally good results in the dog were obtained by passing a glass tube straight down the left carotid from just above the bifurcation.

A further elaboration of this method was devised in cats under light pentobarbital anesthesia, by closing the chest after the cannula had been tied in, and restoring normal respiration. For this purpose the chest was opened about 4 cms. to the left of the midline between the 4th and 5th ribs, and an adequate exposure was obtained by inserting a self-retaining rib retractor and pulling the incised pericardium towards the left. After the left coronary artery had been prepared, the left subclavian artery was approached extrapleurally as it bent round the first rib beneath the pectoral muscles. When the glass tube had been secured in the left coronary artery, the chest was closed in layers and normal respiration restored. The animals appeared in even better condition than those in which the chest was left open.

Perfusion of individual branches of the coronary arteries in the dog. These preparations were used in order to make repeated injections into branches of the coronary arteries without disturbing the heart. The dog was anesthetized with chloralose or pentobarbital and the chest opened under artificial respiration. In order to obtain adequate access to the left coronary artery it was convenient to split the chest open between the 4th and 5th ribs on the left side. The artery to be perfused was then cleaned for a short distance and, after injection of 3–5 mg./kgm. heparin, was connected to either an internal mammary artery, or to one of the common carotids by a flexible rubber tube. Since interruption of the coronary flow for a short while was apt to precipitate ventricular fibrillation, speed was essential in inserting the cannula once the coronary artery had been tied. There was usually a temporary fall of blood pressure and a change in the electrocardiogram, both of which returned to normal within 15 to 30 seconds provided that the flow of blood was restored within half a minute or less.

Figure 2 illustrates the arrangement of cannulae used in comparing the action of veratridine on injection into the right coronary artery (a), the left circumflex artery (b) and the left superior atrial artery (c) which is identical with the ramus atrialis sinister anterior of Meek, Keenan and Theisen (8). The latter arrangement is essentially the same as that described by Smith and Layton (9). There are two minor points of difference. Firstly it should be observed that the left superior atrial artery (labelled Lt. ant. aur. in their fig. 1) usually supplies the left atrium, and very often the right atrium too, as well as the left auricular appendage. Secondly the small length of the left circumflex artery between the cannula and the left superior atrial artery commonly gives off several small twigs (shown in Fig. 2) to the left ventricle. Attempts were always made to tie these twigs, not always successfully since some arise posteriorly; the results were checked at the end of the experiment by injection of a black dye.

Exteriorized heart. This preparation resembles in principle that described by Drinker (10), but differs in some important details. Seven cats weighing from 1.9 to 3.4 kgm. were used, under chloralose (0.08 gm./kgm.) or pentobarbital anesthesia (25 mg./kgm. intravenously after ether induction). Artificial respiration was begun, the pectoral muscles retracted widely and the chest opened, 1 to 1½ cms. to the left of the midline between the 5th and 6th ribs, using electric cautery. The incision was continued 2–3 cms. laterally. If the cats were large enough (3 kgm. or more) this exposure was adequate; otherwise a small portion of the 5th rib was removed after the underlying vessels had been secured by ligatures around the rib. The pericardium was picked up with haemostats, and the anterior medi-

astinum separated from it as far as possible in order to increase the mobility of the heart. A small incision was made in the pericardium in the line of the ribs, and after stay sutures had been inserted at either end to secure it, the pericardium was inverted over the hole in the chest by a continuous suture, ensuring an airtight seal. In order to restore negative intrathoracic pressure, air was sucked out of tho chest with a syringe, artificial respiration was discontinued and the pectoral muscles and skin sewn around the hole. The heart was not displaced from the chest cavity (cp. Drinker) and the exposure was therefore considerably less, yet it was easy to identify the right ventricle, the pulmonary artery and the left ventricle and to inject drugs by means of a fine needle into these cavities without interfering with respiratory movements. It was found that excessive tension on the pericardium caused a fall of blood pressure and the greatest care was taken to avoid this; in the experi-

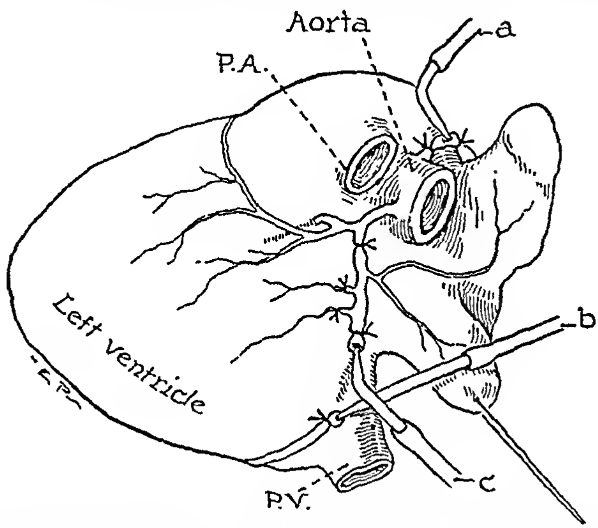


Fig. 2. Left lateral diagram of preparation for simultaneous perfusion of the coronary arteries of the dog. Cannulae are shown in the right coronary artery (a); the peripheral end of the left circumflex (b); and the central end of the left circumflex (c) which now feeds only the left superior atrial coronary artery. The aorta, pulmonary artery (P. A.) and pulmonary veins (P. V.) have been divided.

ments reported the mean arterial blood pressure was from 100–160 mm. of mercury after the preparation had been completed.

Veratridine was chosen for the present investigation not only because it was available in sufficient quantities, but because a considerable amount of preliminary work had already been carried out upon the localization of its site of action, and because the reports suggested that repeated injections in the dosage range required did not elicit responses decreasing in magnitude (tachyphyllaxis) so rapidly as those of some of the other veratrum alkaloids. All doses of the alkaloids refer to the bases. The veratridine was that prepared by Professor R. P. Linstead and Dr. D. Todd, and used by Moe and Krayer in 1943 (11) and subsequently; it had a rotation of $[\alpha]_{Hg}^{22} = +7.7°$ (c = 2.5 in 95% ethanol) and had no sharp melting point, decomposing at 170–178°. The protoveratrine was that prepared by Dr. W. A. Jacobs and used by Krayer, Moe and Mendez (12); it had a rotation of $[\alpha]_{D}^{25} = -8.5°$ c = 1.99 in chloroform) and decomposed at about 273° (uncorr.) after discoloration. The cevadine was prepared by Professor K. P. Link and had a rotation of $[\alpha]_{D}^{22} = +10.7°$ (c = 6.0 in ethanol).

RESULTS. I. *Localization of the principal site of action to the coronary arteries.* 1. *Veratridine.* The following experiments were designed to localize the principal site of action of veratridine more accurately by injection of the drug into the various cavities of the heart. Cats were anesthetized with chloralose or pentobarbital and the chest opened during artificial respiration. The blood pressure was recorded from the left carotid artery and the heart rate from an ink-writing electrocardiograph. In six cats it was found that injection of 5–20 micrograms veratridine into the femoral or jugular veins caused less fall of blood pressure and heart rate, with a longer latency, than when the same dose was injected into the cavity of the left ventricle. Injection of the same quantity of veratridine into the aorta by a fine needle pushed through the wall of the aorta a few mms. above the origin of the coronary arteries had no effect on blood pressure or heart rate. Precisely similar observations were made on three dogs

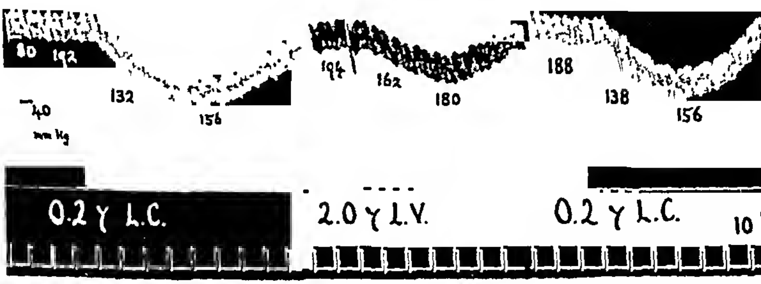


FIG. 3. Dog 5.8 kgm. Pentobarbital. Blood pressure recorded by a membrane manometer from femoral artery; heart rate from ECG in beats per minute indicated by figures below pressure tracing. *Injection of 0.2 micrograms veratridine into the main left* coronary artery (L. C.) causes a greater fall of blood pressure and heart rate than 2.0 micrograms in the cavity of the left ventricle (L. V.). Time in 10 second intervals.

under chloralose anesthesia. These findings strongly suggested that veratridine caused a fall of blood pressure and heart rate principally by its action on some structure in the area of distribution of the coronary arteries.

In order to demonstrate that veratridine evokes a depressor and cardiodecelerator reflex from the coronary arteries, it was injected into the right coronary artery in 8 cats, and into the left coronary artery in 6 cats and 3 dogs with the chest open and in 2 cats with the chest closed. In these animals veratridine caused a fall of blood pressure and heart rate in a dose of from 0.1–0.25 microgram, that is between $\frac{1}{10}$ and $\frac{1}{20}$ of the minimal effective dose injected into the cavity of the left ventricle (fig. 3). These observations warrant the conclusion that the principal receptors of the veratridine depressor and cardio-decelerator reflex are located within the area of distribution of the coronary arteries.

The site of action of veratridine was still further circumscribed by injection into the various branches of the coronary arteries. In 4 dogs injection of 0.25–1.0 microgram veratridine into the left anterior descending coronary artery (either at its origin or up to 4 cms. from its origin) caused a fall of blood pressure

and heart rate, which was abolished by cutting the vagi. The minimal effective dose in these experiments on injection into the cavity of the left ventricle was from 2.5–10.0 micrograms veratridine. The area supplied by this artery, therefore, contains receptors for the reflex.

The septal artery, which arises at the bifurcation of the main left coronary artery into left anterior descending and left circumflex, is inaccessible, but injection into the left circumflex artery caused a fall of blood pressure and of heart rate in more than 20 dogs (figs. 4 and 7). The minimal effective dose was 0.1 microgram veratridine; repeated injections of the same dose gave less and less effect, and the effect was abolished by vagotomy. It was also much reduced by cutting the branches of the vagus which run across the left auricle to reach the left ventricle and the branches which curve under the pulmonary artery at the origin of the left coronary artery to spread over the left ventricle.

In 2 dogs veratridine was injected into large branches of the right coronary artery supplying the right ventricle only (as shown by subsequent injection of a dye). In 6 dogs veratridine was injected into the right coronary artery at or near its origin from the aorta. In none of these experiments did the injection of veratridine have any action on the cardiovascular system, even in doses up to twenty times those causing a large response on injection into the left circumflex artery of the same animal.

In 9 dogs veratridine was injected into the left superior atrial artery (see under Methods). In 5 the veratridine elicited no response even in a dose 20 times the minimal effective dose in the left circumflex artery; in 2 of these the artery supplied the left auricular appendage or the upper portion of the left atrium only as shown by dye injection, in the other three it supplied the upper part of the left and the right atria, reaching up beyond the entrance of the azygos vein into the superior vena cava. In the remaining 4 dogs veratridine caused a fall of blood pressure and heart rate, and in all of these injection of the dye showed that the small portion of the circumflex artery between the cannula and the left superior atrial artery gave rise to vessels supplying the left ventricle which had not been ligated.

The evidence suggests therefore that veratridine elicits in a dose of 0.1–0.25 microgram a cardio-decelerator and depressor reflex on injection into the arterial supply of the left ventricle. The right coronary artery, which supplies by far the greater part of the right ventricle and right atrium, has been eliminated as a possible site of action, as has also the left superior atrial artery. The middle and inferior left atrial arteries are difficult of access for separate perfusion. While it is known from the work of Moore (13) that the left circumflex coronary artery in the dog gives a small supply to the right ventricle, the lower part of the right atrium and (occasionally) the inferior vena cava, as well as to the left atrium, injection of veratridine into the other vessels which supply these structures was without effect, and by far the greater proportion of the blood supplied by this artery goes to the left ventricle. The evidence of these experiments is therefore in favour of the conclusion that veratridine elicits a depressor and cardio-decelerator reflex from the left ventricle. It may be observed in passing

that these injections of veratridine had no effect on the electrocardiogram other than that which would be expected from a reflex slowing of the heart rate.

2. *Cevadine and protoveratrine.* The circulatory actions of the three veratrum alkaloids cevadine, protoveratrine and veratridine are similar in that they have all been shown to cause a fall of blood pressure and heart rate on injection into a dog heart-lung circuit which is left in nervous connection with the separately perfused head; this action is abolished by vagotomy. Their relative activity was therefore compared on injection into the left circumflex artery of three dogs. Like veratridine, both cevadine and protoveratrine caused a fall of blood pressure and heart rate in a dose far smaller than that required on intravenous injection. The minimal effective dose of cevadine was about three times that of veratridine. Protoveratrine, although its minimal effective dose was about the same as that for veratridine, had a much more prolonged action,

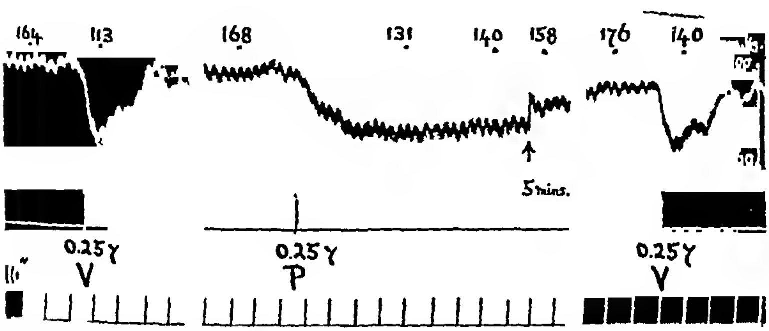


FIG. 4. Dog 7.8 kgm. Pentobarbital. Record of heart rate (beats per minute) above and blood pressure below. Injection of 0.25 microgram protoveratrine (P) into the left circumflex coronary artery causes a more prolonged fall of blood pressure and heart rate than 0.25 microgram veratridine (V). Time in 10 second intervals.

which also reached its maximum more slowly (fig 4). This is in accord with the observations of Krayer, Moe and Mendez (12) on the action of protoveratrine in the intact animal.

3. *Nicotine.* Nicotine in a dose of from 5–50 micrograms injected into the left circumflex coronary artery of dogs also caused a fall of blood pressure and heart rate (fig. 7), which was abolished by cutting the vagi. The analysis of the site of action was not so simple as with the veratrum alkaloids, since injection of the same dose of nicotine into the cavity of the left ventricle had a similar, though smaller, effect. However, the latent period between injection of nicotine into the left circumflex artery and the beginning of the decrease of heart rate (recorded on the ECG) was the same as that observed after injection of veratridine. Injection of the same dose of nicotine into the right auricle or right ventricle had no action on the circulation. By far the greater part of the nicotine injected into the left circumflex artery must have been carried by the coronary sinus to the right auricle; it is therefore justifiable to conclude that

nicotine also elicits a vasodepressor and cardiodecelerator reflex from somewhere in the vascular bed supplied by the left circumflex coronary artery. Injection of 50 micrograms of acetylcholine into the left circumflex coronary artery caused only atrio-ventricular block.

4. *The action of ions.* Following the observation of Szent-Györgyi, Bacq and Goffart (14) that veratrine sensitizes frog striated muscle to potassium ions, Amann and Jarisch (15) suggested that the receptors in the heart were sensitized

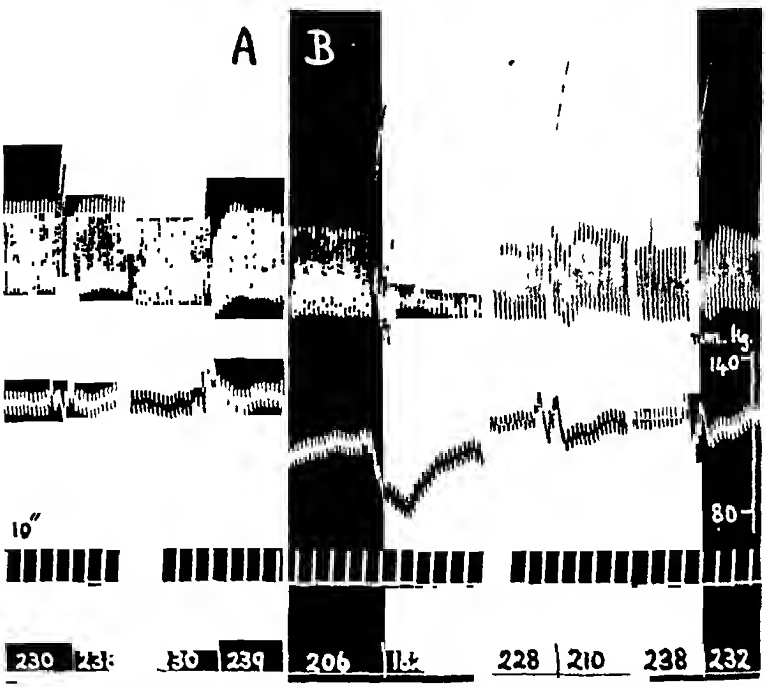


FIG. 5 Cat 2 4 kgm. Pentobarbital Record of respiration by Gaddum's device above, blood pressure below and of heart rate in beats per minute along the baseline At each signal 20 mg potassium chloride 2% was injected into the external jugular vein, at 10 minute intervals. Between A and B 50 micrograms veratridine was injected in divided doses. Time in 10 second intervals

by the veratrum alkaloids in a similar manner. They observed that intravenous injection of 20 mgm. potassium chloride in cats caused a fall of blood pressure and heart rate only when the animals had been given several doses of 0.05–0 1 mgm. per kgm. veratrine hydrochloride previously; this action of potassium ions was prevented by cold block of the vagus or by local anaesthesia of the epicardium. A similar effect was obtained with barium, rubidium, oxalate and citrate; calcium chloride had an antagonistic action. The intravenous injection of such large quantities of these salts in the whole animal might be expected to

have an action upon many other structures besides receptors in the heart. The problem was therefore reinvestigated.

Figure 5 shows that after the injection of 50 micrograms veratridine intravenously into a cat (in divided doses), a previously ineffective dose of 20 mgm. 2% potassium chloride not only causes a fall of blood pressure and heart rate, but also a transitory depression of respiration. Similar effects were obtained with from 5–25 micrograms veratridine per kgm. Since von Euler (16) had observed that much smaller quantities (0.5–1.0 mgm.) of potassium chloride would cause a fall of blood pressure and heart rate, and also respiratory depression, on injection into the central nervous system of cats, it seemed possible that the observed potentiation of potassium chloride might be due in part to an action of veratridine upon the central nervous system. Small doses of isotonic potassium chloride solution were injected into the central nervous system of cats from a cannula tied into the external carotid artery and directed towards the carotid sinus, which was denervated. As figure 6 shows, after the *intravenous* injection of 50 micrograms of veratridine (in divided doses) 0.05 cc. 1.15% potassium chloride injected intra-arterially causes a fall of blood pressure and heart rate, and a transient depression of respiration. Injection of the same dose of potassium chloride into the jugular vein had no cardiovascular or respiratory action. While 5–20 micrograms per kgm. veratridine intravenously usually intensified the fall of blood pressure and heart rate, and the respiratory depression due to the action of potassium chloride on the central nervous system, still larger doses of veratridine reduced or abolished it. This reduction may have been partly due to the prolonged fall of blood pressure (and consequent central anoxia) which is caused by repeated injections of large doses of the veratrum alkaloids. Since the effect of peripheral vagal stimulation upon the heart rate also was not altered in four cats by large doses of veratridine, the potentiation of the action of potassium chloride shown in figure 5 by smaller doses of veratridine is probably due to an action on the central nervous system rather than peripherally.

Though part of the potentiation by veratridine of the action of potassium chloride injected intravenously is attributed to an effect upon the central nervous system, this does not imply that a similar potentiation may not occur in the heart. This possibility was tested by injecting isotonic potassium chloride into the left circumflex coronary artery of dogs, before and after the injection of veratridine. As little as 0.05 cc. 1.15% potassium chloride caused some change in the QRS complex, but no change in heart rate or blood pressure. With doses of 0.1 cc. or more, still greater changes appeared in the electrocardiogram, and in some animals there was a fall in heart rate and blood pressure, which was abolished by cutting the vagi. This dose of potassium chloride would be expected to cause an enormous local increase in the serum potassium (perhaps as much as 3 or 4-fold) and the fact that gross electrocardiographic changes appeared before there was any alteration in heart rate makes the physiological significance of these observations dubious. It may be that such doses of potassium chloride can stimulate nerve fibres in continuity. A previous injection of veratridine into the left circumflex artery, in a dose sufficient to cause a large

fall of blood pressure and heart rate, did not modify the effect of a subsequent injection of potassium chloride. Simultaneous injection of veratridine and a

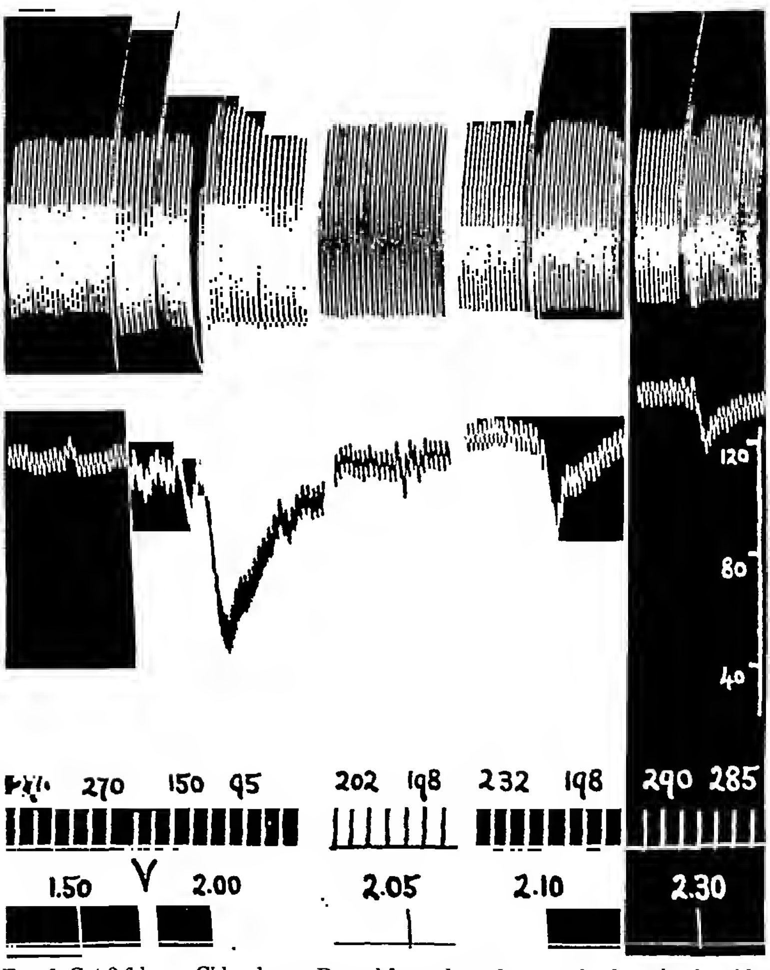


FIG. 6. Cat 2.6 kgm. Chloralose. Record from above downwards of respiration, blood pressure and heart rate (beats per minute). At 1:50, 2:00, 2:10 and 2:30 P.M. injection of 0.05 cc. isotonic potassium chloride into the left internal carotid artery through the denervated carotid sinus. At 2:05 P.M., 0.05 cc. isotonic potassium chloride into the jugular vein. Between 1:50 and 2:00 P.M. the drum was stopped (at V) and 50 micrograms veratridine was injected in divided doses into the jugular vein. Time in 10 second intervals.

minimal effective dose of potassium chloride caused a greater fall of heart rate and blood pressure than either alone; this may have been due to addition of the two effects. These experiments do not lend credence to the view that veratri-

dine acts by sensitizing receptors in the ventricle to potassium chloride, yet it would be rash to dismiss the hypothesis categorically.

Injection of 0.1 cc. isotonic (1.6%) calcium chloride in the same syringe as 0.25 micrograms veratridine into the left circumflex coronary artery of dogs greatly reduced the action of veratridine on heart rate and blood pressure (fig. 7). Sodium citrate (0.1–0.2 cc. of 2.75% solution) increased and prolonged the action of veratridine. Neither of these substances had any cardiovascular action when injected alone in these doses, nor did they modify the action of nicotine injected into the left circumflex coronary artery (fig. 7). The site of action, or perhaps the mode of action of nicotine is therefore different from that of vera-

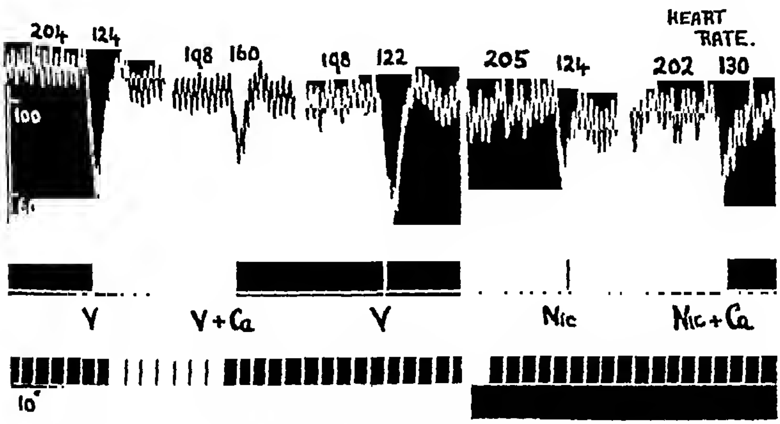


FIG. 7. Dog 7.0 kgm. Chloralose. Record of heart rate in beats per minute and of blood pressure. Injection of 0.25 microgram veratridine and saline (V) into the left circumflex coronary artery causes a greater fall of blood pressure and heart rate than 0.25 microgram veratridine + 0.075 cc. isotonic $CaCl_2$ (V + Ca). The same dose of calcium does not modify the action of 10 micrograms nicotine (Nic). Time in 10 second intervals.

tridine. And there is evidently some close connection between the action of veratridine at this site and the ionic environment, just as there is in striated muscle (5). These tangled relationships have not yet passed the descriptive stage.

II. *The action of veratridine on injection into the perfused lungs.* The evidence presented up to this point has emphasized the importance of the coronary circulation as the principal site of action of the alkaloids veratridine, protoveratrine and cevadine. The experiments of Richter and Amann (4) suggested that in addition these substances might evoke a depressor and cardio-decelerator response arising from the lungs.

In three dogs the left lower lobe of the lung was perfused with defibrinated blood at 37°C. from a pump, and in three further dogs both left upper and lower lobes were perfused. In these six dogs, weighing from 12–23 kgm., injection of 2.5–10 micrograms veratridine into the lung circuit caused a fall of blood pressure and heart rate. This is illustrated in figure 8 which shows that the depressor and

cardio-decelerator action of 5 micrograms veratridine was abolished by cutting the left vagus in an experiment in which both left lobes were perfused, though injection of the same dose of veratridine into the cavity of the left ventricle still caused a fall of blood pressure and heart rate. The action of veratridine injected into the lung circuit was therefore not due to leakage into the rest of the animal; moreover injection of 0.1 mg. epinephrine into the lung circuit failed to cause a rise of blood pressure or heart rate. These experiments demonstrate the ability of veratridine to elicit a depressor and cardio-decelerator response arising from the lung, which probably reaches the central nervous system by the ipselateral vagus.

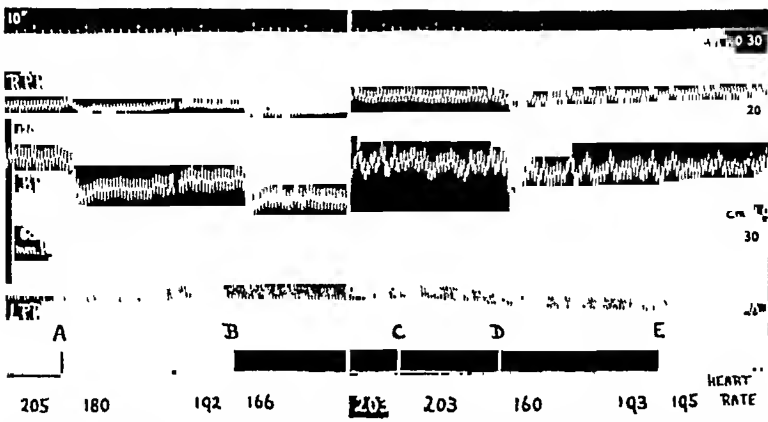


FIG. 8. Dog 13.5 kgm. Chloralose. Whole left lung perfused with defibrinated blood separately from rest of circulation. Records from above downwards of right pulmonary arterial pressure (R. P. P.), blood pressure (B. P.) and left pulmonary perfusion pressure (L. P. P.). Injection into the left pulmonary arterial perfusion cannula of 2.5 micrograms veratridine (A), 5.0 micrograms veratridine (B and C) and 0.1 mgm. epinephrine (E). Between B. and C. the left vagus was cut, and at D 5 micrograms veratridine were injected into the cavity of the left ventricle. Time in 10 second intervals.

The first response to veratridine injected into the lung was always small and subsequent injections of the same dose gave diminishing responses. The largest response observed was a fall of blood pressure from 96 to 74 mms. of mercury and of heart rate from 139 to 90 beats per minute. The minimal effective dose on the lungs (2.5 micrograms veratridine) was more than ten times that required on injection into the left circumflex coronary artery in the dog. Further evidence that the receptor areas in the lungs were of relatively small importance was obtained by using the exteriorized heart preparation, whereby injection could be made into the various cavities of the heart under direct vision without interfering with the animal's normal respiration. Figure 9 is a tracing from such an experiment which shows the much greater fall of blood pressure and heart rate on injection into the cavity of the left ventricle than on injection into the pulmonary artery. Similar results were obtained in five cats under pentobarbital anesthesia.

In a characteristic experiment the electrocardiographic record showed that the latent period before the heart began to slow was from 2.5–4.0 seconds after injection into the left ventricle, and from 8.0–8.5 seconds after injection into the pulmonary artery. The difference between these latent periods would account for the passage of the drug through the lungs and suggests that in the doses used it had little or no action during its transit, contrary to the opinion of Cramer (17).

III. *The action of veratridine on respiration.* Since the experiments of von Bezold and Hirt (1) with veratrine and of Cramer (17) with veratrum viride it has been known that small doses of the veratrum alkaloids cause a transient

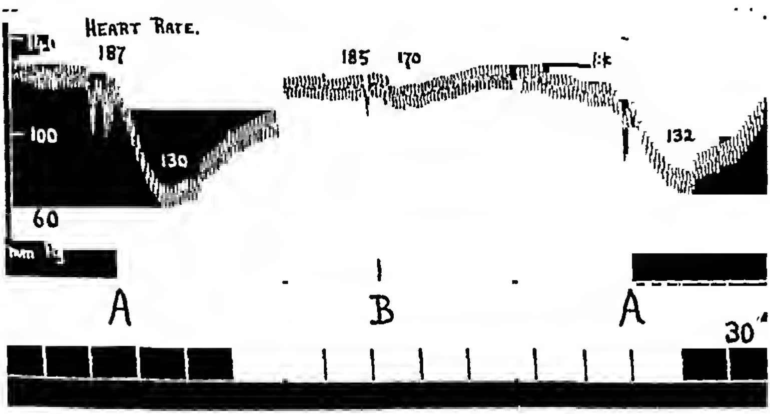


FIG. 9. Cat 2.3 kgm. Pentobarbital. Heart exteriorized, and spontaneous respiration restored by sewing pericardium to chest wall. Record of blood pressure and heart rate (beats per minute). Injection of 10 micrograms veratridine at A into cavity of left ventricle, at B into pulmonary artery. Time in 30 second intervals.

slowing or stoppage of respiration, which is abolished by cutting the vagus or by cold-block of the vagus (3), but which is not modified by doses of atropine which greatly reduce the vasodepressor and cardio-decelerator response (18). The minimal effective dose required to reduce the rate or amplitude of respiration is considerably greater than that required to reduce the blood pressure or heart rate. The respiratory and cardio-vascular actions of the veratrum alkaloids are not therefore mutually interdependent, nor do they necessarily originate by stimulation of the same receptors. The following sites of action may be considered, viz. the lungs, the carotid chemoreceptors—as suggested by Jarisch and Richter (3), the aortic chemoreceptors (which in the cat according to Comroe (19), are supplied by the coronary arteries), and the central nervous system.

To study these alternatives the exteriorized heart preparation was used. Respiratory changes were recorded by a modification of Gaddum's method (20). In six cats under pentobarbital and one cat under chloralose anesthesia injection of 5–10 micrograms of veratridine caused a reduction in the rate and depth of

respiration when injected into the right ventricle or pulmonary artery, but not when injected into the left ventricle. Figure 10 illustrates this difference in an experiment in which the blood pressure responses were not very dissimilar. When the record was taken on a faster drum it was seen that respiration stopped before the blood pressure or heart rate began to fall. The depression of respiration is abolished by vagotomy and is unaffected by atropine. Injection of veratridine into the left coronary artery in two cats in which the chest had been closed

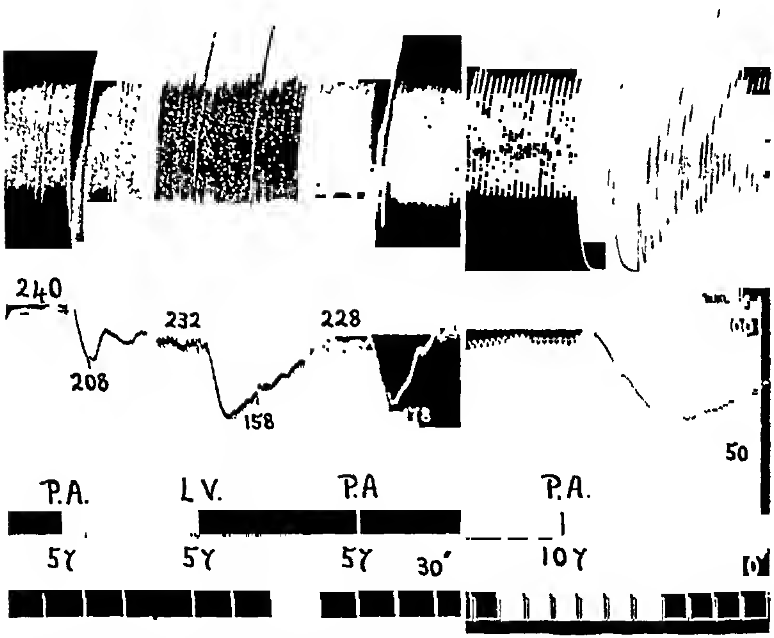


FIG. 10. Cat 2.0 kgm Preparation as in fig. 9. Record of respiration (above), blood pressure (below) and of heart rate in beats per minute (alongside BP tracing). Injection of 5 micrograms veratridine into the pulmonary artery (P. A.) causes a reduction in the rate and depth of respiration, while injection into the cavity of the left ventricle (L. V.) does not. Time in 30 second intervals. On a faster drum after 10 micrograms veratridine the fall of blood pressure begins after respiration has stopped. Time in 10 second intervals.

after cannulation, and in which normal respiration had been reestablished, caused no change in respiration, although there was a profound fall in blood pressure and heart rate. We may therefore conclude that the reflex reduction in the rate and depth of respiration which follows the intravenous injection of small doses of veratridine, originates from receptors in the circulation between pulmonary artery and left ventricle. The reflex fall of blood pressure and heart rate on injection of veratridine into the coronary arteries is not accompanied by any change in respiration (unless the blood supply to the central nervous system is reduced below that necessary to maintain the integrity of the respiratory center).

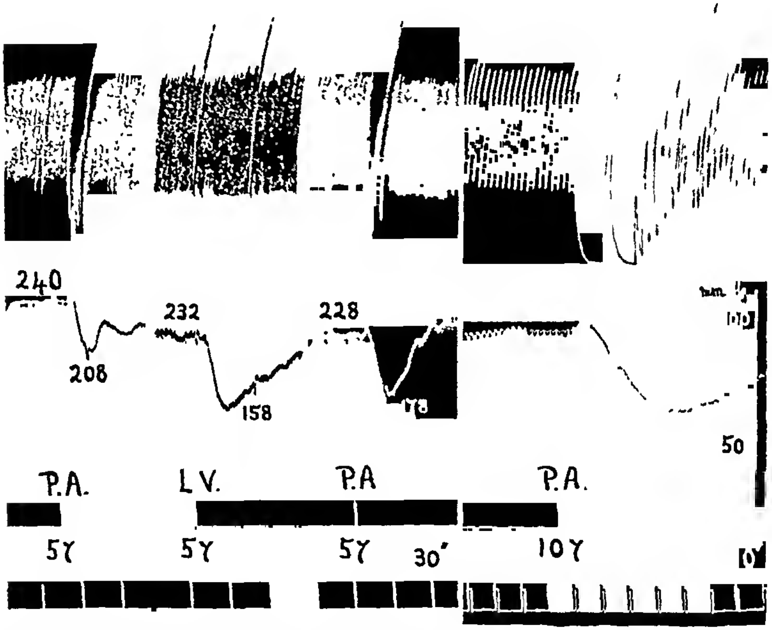
Nicotine injected in a dose of 50 micrograms into the pulmonary artery also caused a small transient reduction in the rate and amplitude of respiration in three out of four cats, which was abolished by vagotomy. This reduction was always followed by stimulation of respiration. Injection of nicotine into the cavity of the left ventricle caused stimulation only. Although the difference was not so striking as that which followed injection of veratridine, it was concluded that nicotine also could evoke a reflex inhibition of respiration from receptors in the lungs.

As Krayer and Acheson (5) point out, the reflex inhibition of respiration is difficult to obtain repeatedly; the receptor mechanism appears to become irresponsive. Further increase in dosage leads once more to respiratory depression, which is independent of the integrity of the vagi. This effect of the veratrum alkaloids is probably due to an effect on the central nervous system. In cats and dogs under pentobarbital or chloralose anaesthesia the carotid sinus was denervated on one side and the external carotid artery ligated. Veratridine was injected into the common carotid artery from a cannula tied into a small side-branch or into the external carotid (pointing towards the carotid sinus); the injected material was thus swept at once into the central nervous system. Injection of 5–20 micrograms of veratridine in cats caused a slowing or stoppage of respiration even when the vagi were cut; this quantity of veratridine represents a far higher concentration of the drug in the central nervous system than would be attained after the injection of the minimal effective dose on the lungs. It is therefore reasonable to conclude that veratridine causes respiratory depression firstly by a reflex originating from the lungs (between pulmonary artery and left ventricle), and secondly by a direct action on the central nervous system.

IV. *The carotid sinus.* Injection of small doses of veratridine into the pulmonary artery of the cat, as has been shown, caused inhibition of respiration, but injection into the left ventricle did not. Injection into the left ventricle caused a fall of blood pressure and heart rate, but injection into the aorta did not. From these observations it was apparent that the carotid sinus was not particularly sensitive to veratridine; it remained to be seen whether it reacted at all. In 1939 Jarisch and Richter (3) reported that injection of veratrine into the exteriorized carotid sinus of the dog caused a fall of blood pressure and heart rate, which was prevented by blocking off the chemoreceptors with lycopodium. This type of experiment was repeated in five dogs, in which the carotid sinuses were perfused with defibrinated blood at 38°C. from the common carotid artery, the pressure being regulated by a Starling resistance on the outflow from the external carotid, and the internal carotid and occipital arteries being tied off. In these five dogs neither veratridine in doses of 1–50 micrograms nor veratrine hydrochloride (Merck) had any action on respiration, blood pressure or heart rate on injection into the perfused sinuses, although there was a good response either to alteration of the perfusion pressure or to injection of cyanide.

Discussion. Jarisch and Richter (3) and Amann and Schaefer (6) have presented evidence to support the theory that the main afferent area of the depressor and cardio-decelerator reflex elicited by the injection of the veratrum alkaloids was in the ventricles. Their evidence was based on experiments in which the cardiac

respiration when injected into the right ventricle or pulmonary artery, but not when injected into the left ventricle. Figure 10 illustrates this difference in an experiment in which the blood pressure responses were not very dissimilar. When the record was taken on a faster drum it was seen that respiration stopped before the blood pressure or heart rate began to fall. The depression of respiration is abolished by vagotomy and is unaffected by atropine. Injection of veratridine into the left coronary artery in two cats in which the chest had been closed



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Schaefer (6) observed in certain cardiac branches of this nerve. In this connection the observations of Daly and Verney in 1926 (22) are relevant. These authors found that raising the pressure in the ascending aorta (the tip of their cannula rested within a few mms. of the coronary orifices) caused a reflex fall of heart rate. They concluded that there were pressure receptors responsible for this reflex either in the coronary arteries or in the left ventricle. The hypothesis that the resistance of the peripheral circulation and the heart rate and output are mutually coordinated by reflexes arising *from the heart itself* both from the inflow chamber (the Bainbridge reflex from the vena cava and right atrium) and from the outflow chamber (the left ventricle) is intriguing. While there is sufficient evidence to justify a discussion of these possibilities, this evidence is still circumstantial; direct proof of the existence of the postulated reflex awaits the solution of great technical difficulties.

Veratridine elicits two reflexes from receptor areas located in the lungs, viz. a fall of blood pressure and heart rate, and a depression of respiration. We do not know whether the receptors for these two reflexes are the same, but we do know that the main afferent paths for both run in the vagi. It is possible that veratridine causes cessation of respiration by stimulation of pulmonary stretch-endings, and that it causes a fall of blood pressure and heart rate by exciting the receptors responsible for the depressor reflex described by Schwiegk (23) and Daly, Ludany, Todd and Verney (24), for which the adequate stimulus appears to be a rise of pressure in the pulmonary vascular bed, probably on the venous side.

SUMMARY

The sites of the cardio-vascular and respiratory actions of small doses of veratridine have been localized by injection into the various cavities of the heart, great vessels and coronary arteries of cats and dogs.

1. By far the greatest part of the reflex fall of blood pressure and heart rate on injection of veratridine originates from the area supplied by the left circumflex and left anterior descending coronary arteries, i.e. the left ventricle. This action of veratridine is increased by the simultaneous injection of sodium citrate and is reduced by calcium chloride.

2. Injection of veratridine into the perfused left lung also causes a fall of blood pressure and heart rate; this area is more than ten times less sensitive than the left ventricle.

3. Veratridine causes a reflex depression of the rate and depth of respiration, originating from the lungs and abolished by vagotomy. In larger doses it causes a similar depression by a direct action on the central nervous system; this is not affected by vagotomy.

4. Injection of veratridine into the perfused carotid sinuses has no effect on circulation or respiration.

5. Veratridine potentiates the action of potassium chloride on the central nervous system in causing a fall of blood pressure and heart rate, and a reduction in the rate and depth of respiration.

branches of the vagus were interrupted, or in which action potentials were recorded from these branches. It is sometimes difficult to be sure that such nerves do not receive twigs from the presso- and chemo-receptors which have been described in and around the great vessels. However, the theory receives substantial support from the experiments described above, which employed an entirely different technique for localizing the site of action. In addition there is reason to believe that the site of action is in the area of distribution of the left circumflex and left anterior descending coronary arteries (in the dog); this may be interpreted as indicating that the left ventricle and the left ventricle only is the site of origin of the reflex.

The possibility that veratridine may convert single responses in nerve fibres into a repetitive discharge cannot be ignored. Acheson (21) has found that injection of veratridine into the circulation of the cat's peroneal nerve causes a single stimulus to give rise to a repetitive discharge; this effect was not easy to obtain and very large quantities of veratridine (0.3 mgm. per kgm.) were required. Nevertheless this consideration should be borne in mind in any discussion of the site of action of the veratrum alkaloids. If this were the explanation of the action of veratridine on injection into the left circumflex coronary artery, one would expect that this alkaloid would also elicit a response on injection into the right coronary artery of the dog or into the carotid sinus; no such response has been observed. Indeed one cannot help being struck by the very unusual distribution of the sites of action of veratridine, unusual that is to say, in terms of the drugs with which the pharmacologist is familiar. It may serve a useful purpose to tabulate the sites of action which have been established so far; viz. (1) it causes a depressor and cardio-decelerator reflex by an action on the lungs, on the region of the left ventricle (the most sensitive area) or on the central nervous system; (2) it causes an inhibition of respiration by an action on the lungs or on the central nervous system; (3) it causes a rise of blood pressure when injected in large doses into the spinal cat; this is attributed to liberation of epinephrine from the adrenals, and is much reduced by cutting the splanchnic nerves or pithing the spinal cord (unpublished observations); (4) sneezing and a burning sensation on contact with veratrine suggest that special sensory receptors may also be excited. In striking contrast, the cardio-vascular and chemo-receptors to which so much attention has been directed in the past two decades do not appear to respond to veratridine. The veratrum alkaloids may therefore serve a useful purpose in drawing attention to receptors and reflexes which are otherwise not readily investigated.

Perhaps the most interesting feature of the depressor and cardio-decelerator reflex elicited by injection of the veratrum alkaloids into the left coronary artery is that its physiological counterpart (if such exists) has not yet been identified beyond dispute. Jarisch, Richter and their collaborators have discussed the possibility that a rise of pressure in the left ventricle might stimulate stretch receptors in the myocardium which would cause a fall of blood pressure and heart rate. The afferent path of this reflex would run in the vagus, and this would account for the discharges in phase with the cardiac cycle which Amann and

THE EFFECT OF BAL AND BAL-GLUCOSIDE THERAPY ON THE EXCRETION AND TISSUE DISTRIBUTION OF INJECTED CADMIUM

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Received for publication January 6, 1947

In a study of the effect of various thiols and dithiols on the course of systemic cadmium poisoning, Gilman and his collaborators (1) have found that delayed treatment with BAL (2,3-Dimercaptopropanol) is moderately effective in reducing the toxicity of intravenous cadmium chloride, but that animals so treated often develop severe kidney damage not produced by cadmium chloride alone. BAL-glucoside was shown to be more effective in treating cadmium intoxication than BAL itself and its use did not produce evidence of renal insufficiency in rabbits. The soluble cadmium BAL complex injected intravenously was even more toxic than an equivalent dose of cadmium as cadmium chloride, but the preformed cadmium BAL-glucoside complex was well tolerated by rabbits in doses equivalent to three times the lethal dose of cadmium chloride. It was believed that some knowledge of the distribution and excretion of injected cadmium and the influence of BAL and BAL-glucoside on its fate might help to explain the mechanism of action of these therapeutic agents. In the experiments to be described the urinary and fecal excretion of cadmium and the cadmium concentration in kidney, liver, and small intestine of rabbits with treated and untreated systemic cadmium poisoning were studied.

Methods. All experiments were performed on rabbits weighing approximately 2 to 3 kilograms. The animals used for tissue analysis were on the standard laboratory diet of Purina dog chow and water. Those used in the excretion studies were fed fresh cabbage and carrots exclusively, to avoid contamination of the excreta with food. They were given increasing amounts of these fresh vegetables in addition to chow and water for 5 or 6 days preceding the experiment. Diarrhea, sometimes produced by an abrupt change to a diet of greens, was thereby avoided in most of the rabbits.

For the excretion studies the rabbits were kept in individual metabolism cages and daily collections of urine and feces were made for five days after treatment. Approximately one-third the 24 hour volume of urine for each animal was used for analysis. The urine sample was evaporated to dryness in a large beaker on a hot plate. The residue was washed into a 30 ml. vycor crucible and again evaporated to dryness. The drying was completed by leaving the crucible overnight in an oven at 110°C. The sample was then ashed in a muffle furnace at 450–500°C. for approximately 48 hours and analyzed for cadmium as described below. It was found in the early experiments that no detectable cadmium was excreted in the urine after the first three days, so the urine collected during the fourth and fifth days was not analyzed subsequently. The total 24 hour collection of feces for each rabbit was dried overnight at 110°C, weighed, and ground to a powder. Aliquots of two to four grams were

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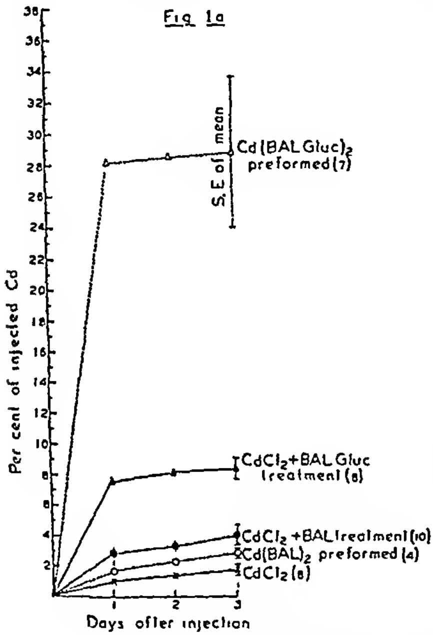
Acknowledgement. I wish to express my thanks to Professor Otto Krayer for his most generous hospitality.

REFERENCES

1. Bezold A. von, and Hirt, L.: Unters. physiol. Lab. Würzburg, 1: 73, 1867.
2. Krayer, O., Wood, E. H., and Montes, G.: This Journal, 79: 215, 1943.
3. Jarisch, A., and Richter, H.: Arch. f. exper. Path. u. Pharmakol., 193: 347, 355, 1939.
4. Richter, H., and Amann, A.: Arch. f. exper. Path. u. Pharmakol., 196: 275, 1940.
5. Krayer, O., and Acheson, G. H.: Physiol. Rev., 26: 383, 1946.
6. Amann, A., and Schaefer, H.: Pflügers Archiv., 246: 757, 1943.
7. Gregg, D. E., and Shipley, R. E.: Amer. J. Physiol., 142: 44, 1944.
8. Meek, W. J., Keenan, M., and Theisen, H. J.: Amer. Heart. Jour., 4: 591, 1928–9.
9. Smith, J. R., and Layton, I. C.: Proc. Soc. Exp. Biol. and Med., 62: 59, 1946.
10. Drinker, C. K.: J. Exp. Med., 33: 675, 1921.
11. Moe, G. K., and Krayer, O.: This Journal, 77: 220, 1943.
12. Krayer, O., Moe, G. K., and Mendez, R.: This Journal, 82: 167, 1944.
13. Moore, R. A.: Amer. Heart. Jour., 5: 743, 1929–30.
14. Szent-Györgyi, A., Bacq, S. M., and Goffart, M.: Nature, London, 143: 522, 1939.
15. Amann, A., and Jarisch, A.: Arch. f. exper. Path. u. Pharmakol., 201: 46, 1943.
16. von Euler, H.: Skand. Arch. f. Physiol., 80: 94, 1938.
17. Cramer, W.: This Journal, 7: 63, 1915.
18. Moe, G. K., Bassett, D. L., and Krayer, O.: This Journal, 80: 272, 1944.
19. Comroe, J. H.: Amer. J. Physiol., 127: 176, 1939.
20. Gaddum, J H.: J. Physiol., 99: 257, 1941.
21. Acheson, G. H.: Personal communication.
22. Daly, I. de B., and Verney, E. B.: J. Physiol., 62: 330, 1926–7.
23. Schwiegk, H.: Pflügers Archiv., 236: 206, 1935.
24. Daly, I. de B., Ludany, G., Todd, A., and Verney, E. B.: Quart. J. Exper. Physiol.. 27: 123, 1937–8.

Throughout this report a difference between two means is considered statistically significant when it is more than three times the standard error of the difference.

RESULTS. *Excretion Experiments.* (Fig. 1) Only a very small part of the injected cadmium chloride was recovered in the urine (fig. 1a) and most of this was excreted during the first 24 hours after injection. Relatively larger amounts were found in the feces (fig. 1b), and the intestinal excretion continued and increased throughout the experimental period, the total cadmium recovered



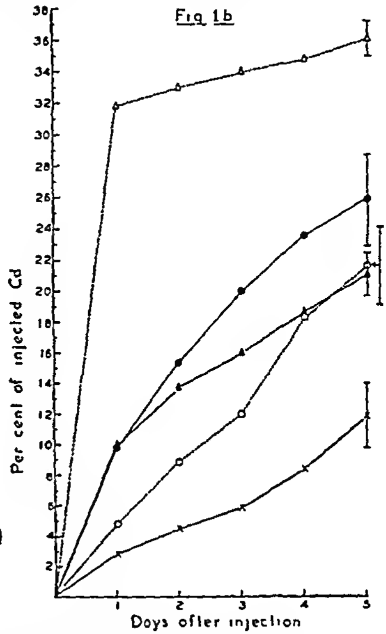


FIG. 1a. CUMULATIVE DAILY URINARY EXCRETION OF CADMIUM

Numbers in parentheses indicate the number of animals in each group. The mean and its standard error are shown.

FIG. 1b. CUMULATIVE DAILY TOTAL EXCRETION OF CADMIUM (URINARY PLUS FECAL)

Symbols as in Fig. 1a.

in the feces (10.1 ± 1.8% of the injected Cd) for the 5 days studied being significantly greater than that in the urine (1.8 ± 0.4%). Treatment with BAL increased the excretion of injected cadmiuam in both urine and feces (fig. 1) especially during the 24 hour period immediately following injection, making the total recovery of cadmium for five days (25.9%) significantly greater than that found for the untreated rabbits (11.9%). When the soluble cadmium BAL complex was injected the excretion of cadmium in the urine was slightly greater than that found for animals given cadmium chloride alone, and the total fecal excretion was about the same as that for the BAL treated group.

taken for analysis. Occasionally the amount of feces produced by a rabbit in a single day was too small for analysis. In such cases the feces for several days were combined and an average value for the 24 hour cadmium excretion was used.

The animals used for tissue analysis were killed by intravenous injection of air at approximately 24 hours (23-25) after the original injection of cadmium chloride or complex. The kidneys and liver were removed , drained of blood, cut into small pieces and dried in petri dishes for about 40 hours at 110°C. A sample of the distal portion of the ileum was removed, washed thoroughly with water, cut up and dried in the same way. Each tissue was then ground to a powder and aliquots of about four grams were analyzed for cadmium.

The rate of excretion of cadmium and its concentration in these three tissues at the end of 24 hours were studied in groups of rabbits given the following types of injections: 1. Cadmium chloride, 2. cadmium chloride and BAL treatment, 3. cadmium di-BAL, 4. cadmium chloride and BAL-glucoside treatment, 5. cadmium di-BAL-glucoside. The structure of the cadmium complexes formed with BAL and BAL-glucoside has been discussed by Gilman (1). In each case the dose per kilogram was chosen so that the cadmium would be equivalent to that in 2.5 mg. anhydrous cadmium chloride. In toxicity tests previously carried out in this laboratory (1) 10 of 12 rabbits given this dose of cadmium chloride survived. All injections of cadmium chloride or preformed complex were intravenous. Treatment with BAL or BAL-glucoside consisted of three injections, each containing 0.1 millimols per kg. of body weight, at 30, 90, and 210 minutes after the cadmium chloride was given. The first of these injections was intravenous, the other two intramuscular. This method of treatment was adopted as a result of the experiments of Gilman and others (1) showing that in the case of BAL such a course of delayed treatment was more effective than prophylactic BAL or BAL given at the same time as the cadmium. Only those rabbits which survived for 24 hours in the tissue studies or for five days in the excretion studies are included in the results.

The BAL used in these experiments was dissolved in propylene glycol (0.5 M solution). All other compounds injected were in physiological saline solution. BAL-glucoside, its cadmium complex, and the cadmium di-BAL complex were prepared immediately before use by methods described by Gilman *et. al.* (1).

Cadmium determinations. The Heyrovsky Polarograph was used to estimate the cadmium concentration in the tissues, urine, and feces. Samples were dried as described above and prepared for polarographic analysis by a method based on that used by Reed and Cummings (2) for determining the concentration of zinc in plants. A sample of dried tissue or feces weighing about four grams was used when possible. The aliquot was weighed into a vycor crucible and dry ashed at 450-500° C. for approximately 48 hours. The procedure for sampling and ashing urine has been described. In each case the ash was treated with four ml. of concentrated hydrochloric acid and evaporated to dryness on a boiling water bath. The residue was dissolved in 2 ml. of 1 N hydrochloric acid, the sides of the crucible washed down with about five ml. distilled water, and the pH adjusted with one per cent ammonium hydroxide to 4-5, using methyl orange as an indicator. The solution was filtered into a 50 ml. beaker, the precipitate washed with 15 to 25 ml. water, and the filtrate and washings evaporated just to dryness on a hot plate. The residue was dissolved in a buffer solution, containing 0.1 M ammonium acetate and 0.25 M potassium thiocyanate, which had been adjusted to pH 4.6 with acetic acid (glass electrode). A drop of 0.1 per cent methyl red solution was added as a maximum suppressor and the solution diluted in a volumetric flask to 10 ml. with buffer. Occasionally some of the residue was not soluble in this volume of buffer, but this was found by recovery tests not to interfere with the cadmium determination when cadmium was present in the amounts found here (10 to 500 microgm.). Nitrogen was bubbled through the solution to remove oxygen and the polarographic determination made. The cadmium concentration was estimated by comparison with a standard curve. The stock standard solution was made from $CdCl_2 \cdot 2\frac{1}{2} H_2O$ and its concentration calculated from a chloride determination. Adequate recoveries of known amounts of cadmium chloride and of the soluble cadmium-BAL complex from tissues and from urine were obtained.

tration of cadmium in the liver and intestine of the treated rabbits was significantly lower than that found in the untreated rabbits as might be expected under these circumstances When the cadmium BAL complex was injected, the cadmium concentration in liver and intestine at the end of 24 hours was approximately the same as that in untreated rabbits. There was, however, a significant increase in the kidney concentration of cadmium (44.4%) although it was not so great as that in the BAL treated animals.

BAL-glucoside. Rabbits treated with BAL-glucoside after cadmium chloride injection appeared to have a somewhat greater concentration of cadmium in their kidneys at the end of 24 hours than did control animals. This difference was not significant statistically and was much less than the increased kidney cadmium after BAL treatment in spite of the much greater renal excretion of cadmium by the BAL-glucosidc treated animals than that found for the BAL treated group. The total recovery of cadmium in both urine and feces at the end of 24 hours was found to be the same with either type of treatment (fig. 1b). The smaller concentration of cadmium in kidney tissue in the BAL-glucoside treated animals can not therefore be attributed to a more rapid removal of cadmium from the body as a whole. Somewhat smaller amounts of cadmium were present in the liver and intestine of the BAL-glucoside treated animals than in the controls, the difference being significant only in the case of the intestine. When the preformed cadmium di-BAL-glucoside was injected, the 24 hour concentration of cadmium in both liver and intestine was significantly lower than that found in rabbits given the same amount of cadmium as the chloride. These results might be expected, since at that time 32 per cent of the administered cadmium had been excreted by rabbits given this complex in contrast to 2.9 per cent by cadmium chloride controls. The concentration of cadmium in the kidney was not, however, significantly lower than that found in the control group.

Discussion. The effects of BAL on the excretion and tissue distribution of intravenously administered cadmium observed in these experiments are, in general, similar to those reported by Gerard and others (3). Somewhat different results have been obtained in treating animals poisoned by cadmium inhalation. In this circumstance the cadmium excretion is shifted in the direction of the kidney, rather than being excreted in larger amounts in both urine and feces (4). No effect of BAL on the tissue distribution of inhaled cadmium was found in dogs by Bunting and his collaborators (5). In such animals BAL is much more effective therapeutically than in animals given cadmium intravenously (4), there being no evident renal pathology in the former group. The mechanisms involved in these differences in distribution and excretion and their relationship to the comparative therapeutic efficacy of BAL when cadmium is administered by the two routes are not immediately apparent.

The toxicity of cadmium and the effects of BAL in cadmium-poisoned animals are believed to involve the same types of reactions that have been described in connection with arsenic poisoning (1). The effect of BAL on the excretion of arsenic has been studied in man (6, 7, 8) and in experimental animals (9, 10). In most instances an increase in the urinary excretion of arsenic during the first

When cadmium poisoned rabbits were treated with BAL-glucoside 7.6% of the injected cadmium was recovered in the urine in the first 24 hours (fig. 1a), much more than that excreted by the BAL-treated animals during the same time (2.9%). However, the 24 hour fecal excretion by the former group was not very different from that in the untreated animals. The total amount of cadmium excreted during the first 24 hours and over the whole five day period studied was approximately the same for animals treated with either compound (fig. 1b). The greater therapeutic value of BAL-glucoside can not, therefore, be explained on the basis of a more effective mobilization and enhanced excretion of cadmium. The preformed cadmium di-BAL-glucoside complex injected at the same cad-

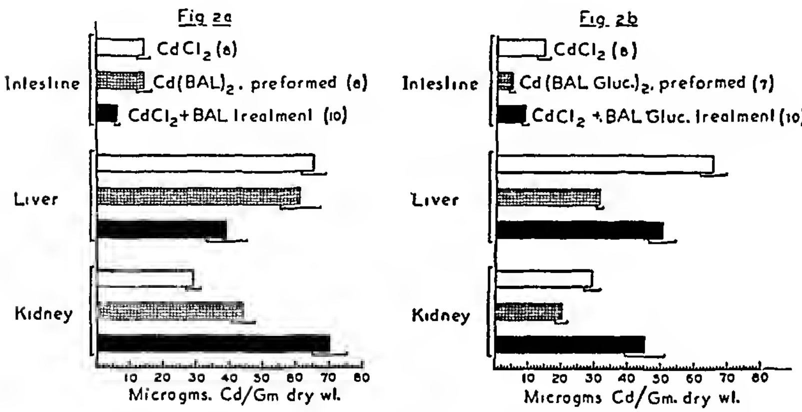


Fig. 2a. Tissue Distribution of Cadmium in Cadmium Chloride, Preformed Cd $(BAL)_2$ and BAL-Treated Cadmium Chloride-Injected Rabbits

Numbers in parentheses indicate the number of animals in each group. Horizontal brackets delimit the standard error of the mean.

Fig. 2b. Tissue Distribution of Cadmium in Cadmium Chloride, Preformed Cd $(BAL\text{-}Glucoside)_2$ and BAL-Glucoside-Treated Cadmium Chloride-Injected Rabbits

mium dose level was excreted in the urine at an extremely rapid rate, 28 per cent of the administered cadmium being recovered in the first 24 hours (fig. 1). The fecal excretion during this period was slightly higher than that found for untreated rabbits, but the difference was not marked.

Tissue Concentration of Cadmium. (Fig. 2) *Cadmium chloride.* Twenty-four hours after cadmium chloride injection, the concentration of cadmium in the liver was significantly greater (65.3 microgm./gm.) than that in either the kidney (28.6) or the intestine (14.3).

BAL. In rabbits treated with BAL after the administration of cadmium chloride there was a marked diversion of cadmium to the kidney, the concentration being increased to 69.7 microgm. per gm. (a statistically significant increase) even when a much larger proportion (9.8%) of the administered cadmium had been excreted than was the case of the untreated animals (2.9%). The concen-

5. The excretion and distribution of cadmium following injections of the soluble cadmium-BAL complex were little different from those found in rabbits given cadmium chloride. When the corresponding complex of BAL-glucoside was injected, comparatively huge amounts of cadmium were recovered in the urine in the first 24 hours after injection.

The author wishes to thank Dr. A. Z. Gilman for suggesting these experiments and for his helpful advice throughout the study.

BIBLIOGRAPHY

1. Gilman, A., Philips, F. S., Allen, R. P., and Koelle, E. S., J. Pharmacol. Exper. Therap., 87, 85, 1946 (supplement).
2. Reed, J. F. and Cummings, R. W., Ind. Eng., Chem. Anal. Ed., 12, 489, 1940.
3. Gerard, R. W., Tobias, J., Potts, A., Lushbaugh, C., Simon, F., Patt, H., Swift, M., Postel, S., and Postelnek, L., N. R. C. C. M. R. report #15, 1944.
4. Tobias, J. M., Lushbaugh, C. C., Patt, H. M., Postel, S., Swift, M. N., and Gerard, R. W.: J. Pharmacol. Exper. Therap., 87, 102, 1946 (supplement).
5. Buntino, H., Harrison, H. E., Ordway, N. K., and Albrink, W. S., N. R. C. C. M. R. Report #11, 1944.
6. Wexler, J., Eagle, H., Tatum, H. J., Magnuson, H. J., Watson, E. B.: J. Clin. Invest. 25, 467, 1946.
7. Leutscher, J. A., Eagle, H., Longcope, W. T., J. Clin. Invest., 25, 534, 1946.
8. Carleton, A. B., Peters, R. A Stocken, L. A., Thompson, R. H. S., and Williams, D. I., J. Clin. Invest., 25, 497, 1946.
9. Eaole, H., Magnuson, H. J., and Fleischman, R.: J. Clin. Invest., 25, 451, 1946.
10. Stocken, L. A., and Thompson, R. H. S., Biochem., J., 40, 548, 1946.
11. Danielli, J. F., Danielli, M., Owen, L. N., and Shaw, G., Military Intelligence Division, Extra-mural Research Report #26, April 21, 1944.

24 hours was reported. As in the case of cadmium, the effect was a short term one, and when the urine was collected at frequent intervals it was shown that the increase in renal excretion was at its maximum 2–4 hours after the administration of BAL. The fecal excretion of arsenic was not studied in most of these experiments. Stocken and Thompson (10) found that BAL treatment did not change the fecal arsenic content of rats poisoned by cutaneous application of lewisite. It is possible that the route of administration of arsenic may influence the effect of BAL on arsenic excretion as it appears to do in the case of cadmium. No studies of the excretion of intravenously injected arsenic after BAL treatment have been reported.

Striking differences in the effects of BAL and BAL-glucoside on rabbits with systemic cadmium poisoning have been reported by Gilman and others (1). The glucoside of BAL had a much greater therapeutic effect than BAL itself in these animals. Animals treated with BAL developed severe renal damage which was not found in the BAL-glucoside-treated rabbits. The results of the experiments reported here correlate well with these observations. A much higher concentration of cadmium was found in the kidneys of the BAL-treated animals than in those of rabbits treated with the glucoside in spite of the fact that more cadmium was excreted in the urine of the rabbits treated with glucoside than was found in the case of those treated with BAL. Danielli and others (11), who first described BAL-glucoside as a therapeutic agent in arsenic poisoning and found it was more effective than BAL, postulated that the differences in action of the two compounds might be explained if BAL glucoside remains extracellular whereas BAL passes into the cell where it is oxidized or otherwise altered. The results of the studies on the treatment of cadmium poisoning with BAL and BAL-glucoside (1) and of those reported here are consistent with this hypothesis.

SUMMARY

1. BAL and BAL-glucoside were found to increase markedly the excretion of cadmium in rabbits poisoned by the intravenous injection of cadmium chloride.

2. BAL treated rabbits excreted larger amounts of cadmium in both urine and feces than did untreated animals poisoned with the same amount of cadmium chloride, whereas the enhanced urinary excretion accounted for almost the entire increase in the BAL-glucoside treated animals.

3. In both groups of animals the increased renal excretion occurred almost entirely in the first 24 hour period following cadmium administration. The fecal excretion of cadmium in BAL treated animals was higher than that for untreated rabbits throughout the five day period studied.

4. A large increase in the concentration of cadmium in the kidney was found in rabbits treated with BAL. There was no significant difference in the cadmium concentration in the kidneys of animals treated with BAL-glucoside from that in untreated rabbits poisoned with cadmium. The concentration of cadmium in small intestine and liver was found to be smaller in animals given either type of treatment than in those with untreated systemic cadmium intoxication.

arsenite injection (table 1). They all showed intense motor excitement and unrest and could be restrained only with difficulty. They cried, rubbed their noses with their forepaws, showed injection of the conjunctiva and reddish discoloration of the iris, constriction of the pupil, salivation, sweating on the lips and slowing of the heart. Most of the rabbits blinked and closed their eyes and showed tear secretion with thick, whitish material. The duration of these symptoms ranges from 6 to 30 minutes. The central excitement only lasts for a few minutes, while the injection of the conjunctiva, the dilatation of the uveal blood vessels, the bradycardia, sweating and salivation and miosis may not disappear for half an hour (figs. 1, 2).

In another series of experiments not listed in table 1, 12 rabbits received a dose of 4.5 mg. of sodium arsenite by vein and 5 minutes later a dose of 2.5 mg. of

TABLE 1

The responses of Albino rabbits to combined administration of BAL and arsenicals

NO OF ANIMALS	DOSE OF BAL (MG/KG BY MUSCLE)	DOSE OF ARSENICALS (MG/KG BY VEIN)	% MIOSIS	HEART RATE (FROM OVER 180/MIN)	SALIVATION & SWEATING	CENTRAL EXCITEMENT
6	20	----------	----	---------	-------	-------
6	--	6-Na arsenite	----	---------	-------	-------
6	15	3-Na arsenite	80	75	++++++	++++++
6	10	5-Na arsenite	75	70	++++++	++++++
6	20	4-Na arsenite	maximal (pin-point)	50	++++++	++++++
6	20	6-Na arsenate	----	---------	-------	-------
3	20	40-arsphenamine	----	---------	-------	-------
3	20	100-neoarsphenamine	----	---------	-------	-------
3	20	10-mapharsen	30	115	+--	++-
4	20	5-$HgCl_2$	----	---------	------- (diarrhea)	-------

BAL suspended in saline solution, also by vein. Following the injection of BAL the typical syndrome with cry, motor excitement, injection of the cornea, miosis, salivation, lip sweating and bradycardia developed. Two rabbits received 1.0 mg. and 2 additional rabbits 5.0 mg. of BAL following the administration of arsenite with identical results. In six animals the intravenous injection of the above doses of BAL alone produced no typical symptoms within 10 minutes.

Premedication with 5.0 mg. of atropine sulfate two minutes prior to the beginning of the experiment failed to prevent the cry, motor excitement, vasodilation and constriction of the pupil, but did prevent the salivation, lip sweating and slowing of the heart. Six rabbits were used for these experiments; 20 mg. of BAL was given by muscle followed 10 minutes later by intravenous injection of 5.0 mg. of sodium arsenite.

Six rabbits were anesthetized with sodium pentobarbital administered by vein in doses of 25 mg. These animals were then given 15 mg. of BAL by muscle,

CENTRAL AND AUTONOMIC EFFECTS FOLLOWING THE COMBINED ADMINISTRATION OF SODIUM ARSENITE AND 2,3-DIMERCAPTOPROPANOL (BAL)

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Received for publication January 7, 1947

Recent studies in this laboratory have shown that arsenite increases the duration and intensity of alcohol narcosis but not of pentobarbital anesthesia (1). This finding was explained in terms of the concept of Peters and associates (2, 3, 4), namely, that arsenite inactivates the pyruvate oxidase system and leads to an increase in the blood pyruvate level in the arsenic-treated animals. While this work was in progress it was thought advisable to counteract the effect of arsenite by the recently introduced chemical antidote, dithioglycerol, or BAL. A number of rabbits, therefore, were treated with BAL prior to the administration of sodium arsenite. Following the combined administration of these two drugs, a peculiar behavior pattern was noted, the analysis of which is the subject of this report.

METHODS. Albino rabbits were used throughout the experiments. BAL (dithioglycerol, 2,3-dimercaptopropanol) was administered either intramuscularly in doses of from 10 to 25 mg. per kg of body weight[1] dissolved in peanut oil, or intravenously in aqueous suspensions in doses from 0.3 to 5.0 mg. Sodium arsenite was always given intravenously in a 1 per cent watery solution in doses from 3.0 to 6.0 mg. The latter was prepared by stirring 10 grams of arsenic trioxide and 6.3 grams of sodium bicarbonate in 100 cc. of boiling distilled water until solution was effected. Upon cooling, a sufficient quantity of distilled water was added to make the solution measure 1,000 cc. Other arsenic compounds used in these experiments were also given by vein in a 1 per cent aqueous solution. Solutions of arsphenamine were prepared in the customary fashion.

All animals were used in the wakeful state, with the exception of one series of experiments in which the rabbits were anesthetized with 25 mg. of sodium pentobarbital given by vein, and their responses to the combined administration of BAL and arsenite noted. A few dogs and cats were also used following the completion of the experiments in rabbits to permit better evaluation of the results obtained in rabbits.

The term, combined administration of sodium arsenite and BAL, requires some explanation since these agents were administered separately from 5 to 15 minutes apart. In the majority of experiments BAL was administered first intramuscularly, followed by an intravenous administration of arsenite or other arsenicals. In a few instances arsenite was given first followed by intravenous injection of BAL.

RESULTS. Table 1 shows that animals receiving BAL alone or arsenic alone under the conditions of this experiment failed to exhibit observable toxic reactions. On the other hand, animals receiving various doses of BAL intramuscularly, and 10 minutes later an injection of sodium arsenite intravenously, developed a set of symptoms immediately following, and sometimes during

[1] All doses are expressed in terms of milligrams of the drug per kilogram of body weight. To avoid repetition, the words "per kilogram of body weight" are omitted.

the heart, but only scant salivation and lip sweating in 3 animals, and none in the remaining rabbits. There was thus a fleeting awakening or denarcotizing effect produced by the combined administration of BAL and arsenite, but the anesthesia (nembutal) diminished the autonomic excitation.

Three dogs and 3 cats were given 15.0 mg. of BAL intramuscularly followed 15 minutes later by intravenous injection of 4 mg. of sodium arsenite. All these animals showed motor excitement, rage to such an extent that it was difficult to immobilize and place them into cages. They showed the same type of autonomic stimulation as rabbits with the notable exception of pupillary constriction and, of course, lip sweating (the latter being characteristic only of rabbits), but showed a phenomenon rabbits failed to exhibit, namely, immediate micturition and defecation. In these species symptoms also disappeared within one hour.

Of the sulfhydryl compounds only BAL was used in this series of experiments, but as table 1 shows, several arsenicals were used in addition to sodium arsenite. Sodium arsenate and a number of organic arsenic compounds failed to produce central excitement and parasympathetic effects when injected following the administration of BAL. The intravenous injection of mercuric chloride was likewise ineffective.

When various amounts of BAL and sodium arsenite were mixed in test tubes at room temperature, white precipitates were produced which disappeared upon standing. Massive doses of the precipitate and of the precipitates dissolved in water were injected intravenously in several rabbits, but no symptoms were seen following such administration. In this connection it may be mentioned that sodium arsenate and several of the organic arsenicals did not produce precipitates when mixed with solutions of BAL in vitro.

Discussion. In the above series of experiments certain facts were presented indicating that 2:3-dimercaptopropanol (BAL) developed in the Department of Biochemistry at Oxford University (2, 3, 4, 5, 6, 7, 8) as a chemical antidote for arsenic compounds plus sodium arsenite produce a syndrome characterized by central excitement and stimulation of the parasympathetic nervous system. The central stimulation, under the conditions of the experiment, often develops into a rage but never into convulsions. It is powerful enough to produce a temporary but definite awakening effect in narcotized animals accompanied by respiratory stimulation. The parasympathetic stimulation resulting from the combined administration of BAL and arsenite is clear cut and manifested by miosis, lacrimation, sweating, salivation, bradycardia, and occasionally by micturition and defecation. Atropine does not antagonize the central effects. It is equally ineffective in preventing the miosis in rabbits which thus may be looked upon as a reaction not due to tectal parasympathetic excitation.

It may be argued that the central effects, as well as the peripheral, are merely additive, representing in part BAL effects and in part toxic symptoms referable to arsenic. As a matter of fact, Modell, Chenoweth and Krop (9) distinguished between slight, mild and severe toxic symptoms caused by progressively increasing doses of BAL in cats. Slight symptoms are represented by blinking, blepharospasm and minimal salivation, mild poisoning by intensification of the above

followed by intravenous injection of 5 mg. of sodium arsenite 10 minutes later. There was an immediate outcry, vigorous motor activity and rubbing of the nose

FIG. 1. RABBIT NO. 25 FROM TABLE 1. NINE MINUTES AFTER THE INTRAMUSCULAR INJECTION OF BAL, (20 MG. PER KG.)

FIG. 2. SAME RABBIT ONE MINUTE FOLLOWING THE INTRAVENOUS INJECTION OF 4.0 MG. PER KG. OF SODIUM ARSENITE. NOTE PIN-POINT PUPIL

with the forepaws. There was every evidence of central excitement subsiding in from 5 to 10 minutes. In addition, the pupils constricted maximally, there was an injection of the conjunctiva with blinking and tear secretion, slowing of

reactions may also be elicited if arsenite is given first and BAL later. These effects, particularly the central excitement, may also be elicited under pentobarbital narcosis.

These effects are opposed or counteracted by atropine only if they are referable to parasympathetic stimulation. Thus, the bradycardia, salivation, sweating, and lacrimation are prevented by atropine but, curiously, the pupillary constriction is not.

Cats and dogs do not develop pupillary constriction as a result of the combined administration of BAL and arsenite, but may show involuntary micturition and defecation.

These phenomena are of relatively short duration and none of the animals observed died as a result of the combined administration of the two agents.

Compounds of BAL and arsenite prepared in vitro do not produce the characteristic biological effects.

Arsenates, arsphenamines and mercurials do not elicit the characteristic central and peripheral effects following the administration of BAL.

Possible mechanisms by which the "explosive" reaction may be brought about are discussed.

REFERENCES

1. Koppanyi, T., Anat. Rec., 94: 35, 1946.
2. Peters, R. A., L. A. Stocken and R. H. S. Thompson, Nature, [illegible]
3. Peters, R. A., and R. W. Wakelin, Biochem. J., 40: 513, 194[illegible].
4. Peters, R. A., H. M. Sinclair and R. H. S. Thompson, Bio[illegible]
5. Thompson, R. H. S., Biochem. J., 40: 525, 1946.
6. Stocken, L. A., and R. H. S. Thompson, Biochem. J., 40: [illegible]
7. Stocken, L. A., and R. H. S. Thompson, Biochem. J., 40: [illegible]
8. Stocken, L. A., and R. H. S. Thompson, Biochem. J., 40: [illegible]
9. Modell, W., M. E. Chenoweth and S. Krop, This Jo[illegible]

symptoms with edema of the conjunctiva, lacrimation and drooling of saliva, and frequently urination. Severe BAL poisoning is characterized by marked ataxia, analgesia, increase in the respiratory rate, hyperexcitability, and occasionally myoclonic convulsions. These authors have also shown that atropinization had no effect on the blinking, blepharospasm and conjunctiva edema produced by BAL, but prevented or markedly reduced salivation and lacrimation. It should be noted that even following intravenous injection the symptoms developed after a latent period of from 5 to 10 minutes and persisted for from 1 to 4 hours. The symptoms of arsenic poisoning do not resemble the clinical picture exhibited by rabbits after the combined administration of BAL and arsenite. It should also be pointed out that the only arsenical that is effective under the circumstances, is arsenite.

It should be noted that the sequence of administration of BAL and arsenite is immaterial; that severe effects, such as ataxia and convulsions, which may develop following the administration of highly toxic doses of each of these two drugs are not produced by the combined administration; and finally, that the reactions develop with a lightening-like rapidity and subside earlier than the mild toxic symptoms produced by either BAL or arsenic alone.

It may be postulated that the "explosive" central and autonomic reactions caused by the combined administration of BAL and arsenite are due to the formation of a new substance in the animal body. This substance may be an intermediate product of a chemical reaction between BAL and arsenite that possesses definite stimulating effects on various parts of the nervous system. It is definitely not the final di-thioarsenite complex formed in the course of the antagonism, for when this substance is prepared in vitro and injected intravenously, it fails to elicit the "explosive" reactions previously described. The short duration of these effects may very well be due to the disappearance of this hypothetical intermediate product, or rather to its conversion to the final di-thioarsenite complex. The other possibility, that the combined administration of BAL and arsenite lead to the mobilization of certain substances (neurohormones?) that produce central excitation and parasympathetic stimulation, cannot be ruled out. The same substance may be responsible for a number of effects, some of which are prevented and others unopposed by atropine. It is well known that certain effects of acetylcholine, for example, are not prevented or abolished by atropinization.

It should be added that the above observed phenomena may have a clinical bearing. It is conceivable that in cases of poisoning with arsenites such as Fowler's solution, the protective action of BAL may be accompanied by unpleasant side effects.

SUMMARY

Rabbits receiving BAL intramuscularly and subsequently sodium arsenite by vein, develop an "explosive" reaction characterized by crying, motor excitement, constriction of the pupils, profuse salivation and lip sweating, lacrimation, bradycardia, injection of the conjunctiva, reddening of the lids and uvea. These

that compounds containing a phenyl group in their structure were most active as anticonvulsants. In accord with this too simple conception, Knoefel and Lehmann (1942) studied 5,5-diphenyl barbituric acid and diphenyl acetylurea as well as some other compounds containing a phenyl group, but did not find any of them to be more actively anticonvulsant than 5,5-diphenyl hydantoin. These latter workers found that large doses of diphenyl hydantoin itself are not only non-hypnotic, as had been noted by Merritt, Putnam and Schwab (1938), but actively produce convulsive phenomena as a direct response effect.

To better establish whether a notable difference in anticonvulsive activity may or may not exist between dialkyl barbitals and phenobarbital, 5-butyl-5-ethyl and 5,5-dibutyl barbituric acids were compared in the present study with 5-phenyl-5-ethyl (phenobarbital) and 5,5-diphenyl barbituric acids. Since 5,5-diphenyl hydantoin itself shows convulsant properties with considerable dosages, the convulsant 5-benzyl-5-ethyl and 5-benzyl-5-butyl barbituric acids were included for evaluation of their anticonvulsive actions. Although the studies of Merritt, Putnam and Schwab (1938) on N-phenyl and N-benzyl derivatives of barbital and our own studies (1946) on ring substituted phenobarbitals failed to demonstrate any marked anticonvulsive activity with such compounds, there were included in the present study certain 5-phenyl-5-alkyl barbituric acids.

Experimental. For the present investigation, commercially available 5-butyl-5-ethyl barbituric acid (neonal) and the sodium salts of 5-phenyl-5-ethyl barbituric acid (phenobarbital-sodium) and of 5,5-diphenyl hydantoin (dilantin) were used. The other required derivatives for this work were synthesized by Dr. Roland N. Icke and Dr. C. Ernst Redemann, in Pasadena. The 5,5-diphenyl barbituric acid was prepared by condensation of alloxan with benzene by following the method of Barnes and McElvain (1937). The other derivatives were prepared by developing proper methods for condensing the properly disubstituted malonic or cyanoacetic acid esters with guanidine carbonate in a sodium ethylate solution, giving the corresponding imino barbituric acids which were then acid hydrolyzed to give the desired disubstituted barbituric acids. The following compounds have not been previously described in the literature and gave the following analyses as evidence of identity and purity.

5-Phenyl-5-butyl barbituric acid		
	Found:	C, 64.52; H, 6.57; N, 10.83 %
	Calculated:	C, 64.52; H, 6.25; N, 10.77 %
5-Phenyl-5-*iso*butyl barbituric acid		
	Found:	C, 64.60; H, 6.10; N, 10.73 %
	Calculated:	C, 64.52; H, 6.25; N, 10.77 %
5-Phenyl-5-amyl barbituric acid		
	Found:	C, 65.50; H, 6.58; N, 10.20 %
	Calculated:	C, 65.70; H, 6.62; N, 10.22 %

The other derivatives were identifiable because of their mode of synthesis and comparison of their melting points with those reported in the literature. Their melting points were often a little higher than those reported in the literature, probably because of better crystallization away from impurities.

COMPARATIVE CENTRAL DEPRESSANT ACTIONS OF SOME 5-PHENYL-5-ALKYL BARBITURIC ACIDS

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Received for publication January 11, 1947

INTRODUCTION

The hypnotic actions of a considerable number of 5,5-dialkyl derivatives of barbituric acid have been studied in attempts to relate the chemical constitution of these compounds to their physiological action. Some of the earlier work with certain series of compounds led to the making of broad generalizations with regard to hypnotic effectiveness of barbituric acid derivatives. Thus, Shonle (1931) summarized previous studies of dialkyl derivatives having dissimilar alkyl groups to conclude, for example, that "in the more effective compounds, the sum of the carbon atoms on the two substituent groups was seven." Not only are exceptions to this rule now known, but Butler and Bush (1942) found none of such previously advanced rules to apply to the relative hypnotic effectiveness of the series of dialkyl derivatives that they studied. It is difficult to include the observation of Swanson (1934) that a compound like 5-(1,3-dimethyl-butyl)-5-ethyl barbituric acid in warm blooded animals is not only without effect but acts as a powerful convulsant, in any generalizations about the hypnotic effectiveness of dialkyl derivatives. Pursuing the matter of the unpredictable appearance of convulsive effects among series of these compounds, Knoefel (1945) has found examples of such convulsive effect apparently having some relation to the introduction of an unsaturated bond into the structure of the molecule and to the conversion from a barbiturate to a thiobarbiturate.

The discovery and development of the anticonvulsive effects of 5,5-diphenyl hydantoin by Merritt, Putnam and Schwab (1938) showed that hypnotic and anticonvulsant activities are not necessarily related in the actions of compounds having the hydantoin type of structure. With barbituric acid derivatives a differentiation of these two types of activities was not apparent in this work, for with pentobarbital and *iso*amyl-ethyl barbituric acid small increases in convulsive threshold were associated with dosages that produced mild hypnosis and with phenobarbital hypnotic dosages were required to cause any marked rise in the convulsive threshold. More recent studies of the anticonvulsive properties of these barbituric acids and other compounds by Tainter and coworkers (1943) led them to the conclusion that there was remarkably little difference between the dialkyl barbituric acids and phenobarbital when compared in corresponding dosages.

With regard to chemical typing of compounds used in their investigations, Merritt, Putnam and Schwab (1938) were particularly concerned with the finding

found necessary to substitute for the resistors in the timer circuit some fixed ½ watt resistors of appropriate values to provide the desired time intervals. By this simple modification of the instrument the following shock durations were made available: 0.05, 0.1, 0.2, 0.4, 0.9, 1.8, 3.6, 7.5, 15.0 and 30.0 seconds.[1]

For routine testing the convulsant threshold was taken as the shortest duration at which a 50 milliampere current resulted in a noticeable tonic component in the convulsive response. The initial threshold for the individual rabbit was determined on the day of the test by applying a current of the fixed intensity for 0.05 second. If no tonic convulsion resulted, 15 minutes later the current was applied for 0.1 second, and so on with progressively longer durations at 15 minute intervals until the threshold was reached. The initial threshold was found to be quite constant both for the individual and between individuals. In 426 determinations made on 36 rabbits the initial threshold was found to be 0.18±0.006 seconds with a range of 0.05 to 0.9 seconds. One half hour after the threshold tonic convulsion was obtained the animal received an intraperitoneal injection of the compound to be tested and one hour after this the threshold was again determined by applying the 50 milliampere current for increasing durations from 0.05 seconds to a time value which resulted in a second tonic convulsion. A 15 minute interval between shocks was maintained throughout since investigation showed that shorter intervals were apt to be accompanied by a more or less significant depression resulting from the previous shock, whereas intervals of 15 minutes or longer were found to result in a response free from such depression. By comparing the convulsant threshold following medication with the threshold ½ hour before the injection of the test compound, the anticonvulsant activities of the several compounds were established.

In each instance the compound was injected intraperitoneally in the form of a solution of its sodium salt in water. The compound was taken into solution with a slight excess of sodium hydroxide, then the pH was adjusted to the lowest level possible (between 9.0 and 11.0) which would permit the compound to remain completely in solution, then made up to volume with distilled water. Dosage used was based on and expressed in terms of millimols per kilogram of body weight of the animal (mM/kg).

In order to establish that the depression which follows a tonic convulsion is not of sufficient magnitude to influence the results obtained by administering the drug, each animal was "standardized" by redetermining the time duration threshold without medication at 1½ hours after the initial convulsion. This value for the 33 animals so tested was found to average 0.24±0.023 seconds with a range of 0.05 to 0.4 seconds, or about 30% higher than the initial value. For evaluation of the anticonvulsant activity of the compounds a threshold time increase of 500% or 5 times the initial threshold can conservatively be insisted upon in order that the comparative evaluation of the several compounds may be unquestionable.

Hypnotic and lethal activities of the compounds have been determined in white mice and to some extent in rabbits. In mice, hypnosis was considered positive if righting reflexes were lacking when the animal was placed on its back, and in rabbits the end point was taken to be the inability to stand and the showing of little response when tapped briskly on the rump. In computing the hypnotic and lethal dosages the statistical method of Bliss (1938) was used throughout.

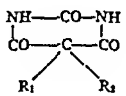
Anticonvulsant activities. The 12 compounds studied were found to fall into three distinct groups, as can be noted by study of table 2. Only 4 of the compounds showed marked anticonvulsant activity. These were, in order of greatest activity at a dosage of 0.2 mM/kg.: 5-phenyl-5-butyl barbituric acid (79 times the original threshold); 5-phenyl-5-propyl barbituric acid (59 times the initial

[1] We have observed that with prolonged application of current, a more or less marked depression becomes evident, and that this becomes more pronounced with the longer durations. With shocks up to 1 second the convulsant action seems to be enhanced, but with durations of 10 seconds or longer, the convulsive response is distinctly less severe.

METHODS. The methods used by the several investigators of the anticonvulsive activities of compounds in recent years have been widely divergent, and the results found have been contradictory in nature in some instances. Before 1937, and frequently since then, the experimental induction of convulsions in animals has been accomplished by injection of one of the several convulsant drugs into the animal, for example, camphor, cocaine, metrazol, picrotoxin, thujone and similar substances. The use of electrically induced convulsive responses in the study of anticonvulsant drugs, first reported by Albertoni (1882), has become common since the introduction of electroshock as a therapeutic procedure in psychiatry by Cerletti and Bini (1938). Speigel (1937) determined the convulsive reactivity of cats and rabbits by electrical stimulation through the eyeballs with an apparatus which permitted variation of both voltage and duration of an alternating current. Merritt, Putnam and Schwab (1938), Knoefel and Lebmann (1942), and Tainter and coworkers (1943) determined the convulsant threshold in terms of current strength with the duration of current flow kept constant at 10 or 15 seconds. On the other hand, Kozelka, Hine and Grieber (1942) determined the convulsant threshold in terms of duration of current flow with the current kept at a constant strength, and by use of this method they were able to show threshold rises of from 2 to 10 times, while the other investigators found increases in threshold of only 1.3 to 2 times the initial level by keeping the duration constant and varying the current intensity.

TABLE 1

Identification of derivatives

		MELTING POINT FOUND	MELTING POINT REPORTED
		°C.	°C.
Butyl	Butyl	155–156	153[a] 158[b]
Phenyl	Propyl	193–194	190[c]
Phenyl	Butyl	213–214	—
Phenyl	*iso*Butyl	175–176	—
Phenyl	Amyl	171–172	—
Phenyl	Hexyl	159–160	152–155[d]
Phenyl	Phenyl	296	290–292[e]
Benzyl	Ethyl	211–212	206–207[f]
Benzyl	Butyl	195	195[f]

[a] Tiffeneau: Bull. soc. chim. 33, 183, (1923).
[b] Kamm and Volwiler: U. S. #1,331,712 Feb. 24, 1920.
[c] Hoerlein: U. S. #1,025,526 May 7, 1912.
[d] Chamberlin, Doyle and Spaulding: J. Am. Chem. Soc. 57, 352, (1935).
[e] McElvain: J. Am. Chem. Soc. 57, 1303, (1935).
[f] Dox and Yoder: J. Am. Chem. Soc. 44, 1141, (1922).

In the present study the following modification of Kozelka's method has been used: The convulsive seizures were produced in rabbits by passing a 50 milliampere 60 cycle sine wave current through the head of the animal for periods of current flow ranging from a minimum of 0.05 second to as long as 30 seconds when that long a duration was found necessary. The current was applied through ¾ inch square solder covered copper electrodes held in position on the temporal region by elastic tapes. Contact was assured by using an electrode jelly. An Offner electroshock therapy apparatus was used, and because of the desirability of using shocks of durations falling between 1 and 10 seconds, as well as above 10 seconds, it was

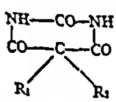
threshold); 5-phenyl-5-ethyl barbituric acid, or phenobarbital, (58 times the initial threshold); and 5-phenyl-5-*iso*butyl barbituric acid (56 times the initial threshold). The minimal effective dose, taken as the dosage that will raise the threshold 500% or 5 times the initial threshold in 50% of the animals, has been computed for these four compounds and for 5,5-diphenyl hydantoin (dilantin) for comparison, using the statistical method of Bliss (1938) and the data is given in table 3.

A second group of compounds showed only moderate anticonvulsive activity at a dosage of 0.2 mM/kg. and included dilantin (8.2 times the initial threshold); 5-phenyl-5-amyl barbituric acid (7.5 times the initial threshold); 5-benzyl-5-ethyl barbituric acid (7.1 times the initial threshold); and 5-butyl-5-ethyl barbituric acid (6.2 times the initial threshold).

The remaining compounds were essentially inactive at a dosage of 0.2 mM/kg. These were 5-benzyl-5-butyl barbituric acid (2.4 times the initial threshold); 5,5-dibutyl barbituric acid (1.9 times the initial threshold); 5-phenyl-5-hexyl barbituric acid (1.1 times the initial threshold); and 5,5-diphenyl barbituric acid (1.3 times the initial threshold). This latter compound at a dosage of 0.5 mM/kg., exhibited a moderate anticonvulsant activity.

TABLE 3

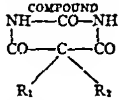
Anticonvulsant activities of barbituric acid derivatives and dilantin

	NUMBER OF RABBITS SHOWING 5 TIMES INCREASE IN DURATION THRESHOLD/NUMBER ANIMALS TESTED					ANTICONVULSANT DOSE AC-50 mM/KG
	Dose in mM/kg					
	0.025	0.05	0.10	0.20	0.40	
Phenyl Ethyl	—	9/23	19/31	27/31	—	0.069 ±.012
Phenyl Propyl	—	4/13	11/20	12/12	—	0.083 ±.016
Phenyl *iso*Butyl	—	6/11	5/11	11/11	—	0.07
Phenyl Butyl	4/12	4/21	18/23	18/20	4/4	0.059 ±.012
Dilantin	—	4/11	5/21	11/21	—	0.279 ±.418

The marked anticonvulsant action of dilantin reported by Merritt, Putnam and Schwab (1938) and by Knoefel and Lehmann (1942) in cats has not been observed in the present investigation in rabbits. At the highest dosages used, 0.2 mM/kg. or 42.2 mg/kg., the threshold was raised by only about 8 times. Moreover, at that dosage severe symptoms of diarrhea were almost always present which lasted for about a week. In three cases death resulted within two days following dilantin intraperitoneally and in two of these the drug was certainly contributory to death, while in the other it was possible that other factors may have entered into the fatal outcome. It seems likely that rabbits are less able to tolerate the strongly basic solution than are cats. Rabbits also seem to be somewhat more resistant to showing anticonvulsant action of the drugs. It is possible that chronic dosage would have shown a greater anticonvulsant effect, but the effect of a single administration only was made the subject of the present study.

TABLE 2

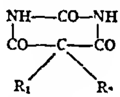
Anticonvulsant activities of barbituric acid derivatives and dilantin on threshold for electrically induced convulsions in rabbits

COMPOUND 	DOSE IN mM/KG	NUMBER OF ANIMALS INJECTED	NUMBER SHOWING HYPNOSIS	NUMBER OF DEATHS	MEAN DURATION IN SECONDS OF 50 MILLIAMPERES CURRENT TO CAUSE TONIC CONVULSION	
					Initial	Final
Control	—	33	0	0	0.18	0.24
Butyl Ethyl	0.05	9	0	0	0.13	0.54
	0.10	9	0	0	0.18	0.77
	0.20	16	11	0	0.16	1.25
Butyl Butyl	0.20	5	3	0	0.12	0.49
Phenyl Ethyl	0.05	23	0	0	0.15	0.63
	0.10	31	0	0	0.16	3.40
	0.20	31	9	0	0.18	11.74
Phenyl Propyl	0.05	13	0	0	0.23	0.66
	0.10	20	0	0	0.19	1.30
	0.20	12	4	0	0.21	11.78
Phenyl *iso*Butyl	0.05	11	0	0	0.17	0.56
	0.10	11	0	0	0.15	0.94
	0.20	11	0	0	0.15	11.22
Phenyl Butyl	0.025	12	0	0	0.17	0.58
	0.05	22	0	0	0.26	0.59
	0.10	23	0	0	0.22	4.00
	0.20	20	0	0	0.20	15.87
	0.40	4	1	0	0.10	24.38
Phenyl Amyl	0.10	8	0	0	0.25	0.32
	0.20	6	0	0	0.21	1.50
Phenyl Hexyl	0.10	4	0	0	0.20	0.65
	0.20	4	0	0	0.26	0.27
Phenyl Phenyl	0.20	7	0	0	0.13	0.38
	0.50	8	0	0	0.17	1.46
Benzyl Ethyl	0.05	3	0	0	0.16	0.30
	0.10	6	0	0	0.13	0.40
	0.20	9	4	0	0.13	1.43
Benzyl Butyl	0.20	8	1	1	0.12	0.22
Dilantin	0.05	11	0	0	0.15	0.55
	0.10	21	0	0	0.19	0.84
	0.20	21	0	3	0.17	1.63

at 0 8 mM/kg. in 59% as calculated from table 4. Calculation of hypnotic dose for 50% (HD-50) from these data gives about 0.7 mM/kg for 5-phenyl-5-butyl barbituric acid, which may be compared with 0.3 mM/kg for 5-phenyl-5-ethyl barbituric acid (phenobarbital) as reported by Shonle and Moment (1923) and confirmed by our data.

TABLE 5

Acute toxicity and hypnotic action of barbituric acid derivatives and dilantin in Albino mice following intraperitoneal injections

	NUMBER OF MICE INJECTED	HD 50 mM/KG	CD 50 mM/KG	LD 50 mM/KG
Butyl Ethyl	50	374 ± 036		1 506 ± 102
Butyl Butyl	50	.321 ± 025		967 ± 048
Phenyl Ethyl	160	277 ± 012		954 ± 042
Phenyl Propyl	140	336 ± 020		1 122 ±.020
Phenyl *iso*Butyl	80	303 ± 033		978 ±.082
Phenyl Butyl	120	333 ± 024		901 ± 010
Phenyl Amyl	140	459 ± 041		1 301 ± 034
Phenyl Hexyl	60	not hypnotic		639 ±.162
Phenyl Phenyl	140	1 362 ± 034		2 040 ±.068
Benzyl Ethyl	60	about .2	about 15	295 ± 094
Benzyl Butyl	60	not hypnotic	308 ± 024	372 ± 023
Dilantin	30	not hypnotic	about 40	.985 ± 103

Some relation among the compounds reported in table 5 is more evident between chemical constitution and hypnotic activity in mice The activity in the series of 5-phenyl-5-alkyl barbituric acids can be expressed as

$$\text{Ph-Et} > \text{Ph-Pr} = \text{Ph-Bu} > \text{Ph-Am} > \text{Ph-Hex}$$

and phenyl-*iso*butyl is almost as active as phenyl-ethyl Butyl-ethyl is somewhat less active than dibutyl, and diphenyl is even less active as an hypnotic agent. The 5-benzyl-5-ethyl derivative showed a marked depressant action, but whether its action is hypnotic and similar to that of the others is not clear because of its convulsant actions

Convulsant activities. Benzyl-ethyl and benzyl-butyl barbituric acids were both found to be convulsant compounds, as had been reported by Shonle and Moment (1923) for the benzyl-ethyl and benzyl-propyl compounds However, the convulsive seizures produced by the two compounds studied are markedly different. Benzyl-ethyl barbituric acid produces a generalized clonic convulsive effect which is recurrent for hours[2] The movements in the convulsion are very

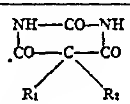
[2] The dose-effect curve for convulsant activity following injections of 5 benzyl-5 ethyl barbituric acid is very steep At 0 1 mM/kg no convulsions resulted, yet at 0 2 mM/kg. all of the animals exhibited convulsive responses This may indicate an all or none type of response with but little variation in threshold between individuals With higher doses

Dilantin is precipitated from a solution of its sodium salt at a pH of 9 or less, and the poor solubility of the free acid might account for the lack of response to a single intraperitoneal injection. It seems doubtful that this can entirely explain the lack of anticonvulsant activity following a single dose in view of the definite convulsant response which can be elicited with only slightly higher single doses A similar problem is encountered with some of the less soluble barbitals used, which also are precipitated at pH 9 and so will be in the form of the free acid at the pH of the body.

Hypnotic Activities The hypnotic actions of the series of compounds included in the present study are summarized for rabbits in tables 2 and 4, and are summarized for mice in table 5 after the statistical computations were made on the

TABLE 4

Hypnotic action of barbituric acid derivatives and dilantin in rabbits following intraperitoneal injections

	NUMBER OF RABBITS SHOWING HYPNOSIS/NUMBER OF RABBITS TESTED								
	Dose in mM/kg								
	0 025	0 05	0 10	0 20	0 40	0 50	0 60	0 80	1 60
Butyl Ethyl		0/9	0/9	11/16					
Butyl Butyl				3/5					
Phenyl Ethyl		0/23	0/31	9/31	2/2				
Phenyl Propyl		0/13	0/20	4/12					
Phenyl *iso*Butyl		0/11	0/11	0/11					
Phenyl Butyl	0/12	0/24	0/25	0/21	2/10		3/7	4/7	7/7(4*)
Phenyl Amyl			0/8	0/6					
Phenyl Hexyl			0/4	0/4					
Phenyl Phenyl				0/7		0/8			
Benzyl Ethyl		0/3	0/6	4/9					
Benzyl Butyl				1/8(1*)					
Dilantin		0/11	0/21	0/21(3*)					

* Number in parentheses represents the number of animals which died.

observations. With the criterion for hypnosis set at a level in rabbits at which the animal is no longer able to sit upright without vigorous prodding, it is to be observed in table 4 that at 0.2 mM/kg the 5-butyl-5-ethyl and 5,5-dibutyl barbituric acids showed hypnosis in 69% and 60% of the animals respectively. Phenobarbital, or 5-phenyl-5-ethyl barbituric acid, showed hypnosis in 29% and 5-phenyl-5-propyl barbituric acid in 33%. In the 5-phenyl-5-alkyl series, with alkyl groups larger than propyl, the hypnotic activities at this dosage were not sufficient to meet the arbitrary criterion for hypnosis. Although none of the animals was down, a 0.2 mM/kg. dosage of 5-phenyl-5-*iso*butyl barbituric acid caused marked interference with equilibrium or obvious drowsiness in 36%, while 5-phenyl-5-butyl barbituric acid was not hypnotic at all. The 5-phenyl-5-butyl barbituric acid was hypnotic at 0.4 mM/kg. in only 20% of the animals and

studied the influence of electroshock on the electroencephalogram in rabbits, using a 60 cycle alternating current of 25 to 35 volts with a duration of 0.1 second. As stated by Jessner and Ryan (1941), "when compared to the electroencephalograms taken during metrazol convulsions the records bore a close resemblance. The brain, once induced to discharge its energy explosively, seems to act in the same way, regardless of how the explosion is set off, and it appears that the pattern is the same for rabbit, cat and man." The anticonvulsant action of drugs must involve a mechanism which increases the resistance against irradiation of motor discharge. Just how this takes place is not clear but it apparently does not necessitate a marked depression of higher nervous centers that are productive of hypnotic action.

The demonstration by Merritt, Putnam and Schwab (1938) that 5,5-diphenyl hydantoin (dilantin) is notably anticonvulsant, yet lacks hypnotic activity, showed the necessity for formulating a new hypothesis as to the mechanism of the anticonvulsant action of hydantoin derivatives and possibly of other types of compounds. The extensive clinically successful use of dilantin for the treatment of epilepsy and related disorders in man, in doses not producing significant degrees of hypnotic action, has well substantiated the idea of differential central depressant actions for dilantin. By use of combinations of phenobarbital and amphetamine (benzedrine) in the treatment of epilepsy, Cohen and Myerson (1938) were able to demonstrate that the hypnotic action of phenobarbital could be effectively antagonized by amphetamine without apparent decrease in effectiveness for the treatment of epilepsy.

In the present investigation a number of observations support the view that two discrete mechanisms are involved in the anticonvulsant and hypnotic actions of the barbituric acid derivatives studied. Action on one of these mechanisms results in hypnosis and action on the other mechanism results in blocking the spread of nervous impulses, which if left unblocked would result in convulsive movements. That phenyl-butyl barbituric acid shows a markedly greater anticonvulsant action than phenyl-ethyl, phenyl-propyl or phenyl-*iso*butyl barbituric acids, while showing an hypnotic activity of only about ⅓ that of phenyl-ethyl or ½ that of the other two compounds, is in accord with such a concept. Butyl-ethyl barbituric acid (neonal) at 0.2 mM/kg. produced sleep in 30% of the rabbits, but only increased the threshold to electrically induced convulsions to 6 times the control level. In contrast, a dose of 0.8 mM/kg. of phenyl-butyl barbituric acid was required to produce sleep in 57% of the rabbits, yet at one-quarter that dosage, without any hypnotic effect, the convulsant threshold was increased to 79 times the control level. That the basic mechanisms for anticonvulsant and hypnotic actions are identical or interdependent is also made very improbable by the presence of both convulsions and hypnosis at the same time following administration of benzyl-ethyl barbituric acid.

The present investigation shows that the presence of a phenyl group in barbituric acid derivatives is important for the development of a considerable degree of anticonvulsant activity. The anticonvulsant activity of the butyl-alkyl derivatives studied is relatively slight. Nevertheless, the presence of a second

rapid. The animal does not usually roll over on its side but drops where it is. The hind legs in particular are in an exaggerated state of extension. The respiratory muscles are not considerably involved and respiration continues quite well during the seizure. Reflex excitability is markedly exaggerated and the slightest touch is sufficient to set off a new convulsive seizure. On the other hand, depression and a kind of hypnosis are evident throughout and the animals apparently die of exhaustion.

In comparison, 5-benzyl-5-butyl barbituric acid appears to be more strychnine-like in action. The convulsive action is tonic rather than clonic and death results from the asphyxia produced by the tonic immobilization of the respiratory muscles. Autopsies performed within a short time after cessation of respiration showed the heart to beat for some time after the respiratory arrest.

Dilantin was likewise found to be convulsant at 0.4 mM/kg. in mice. The convulsions, although clonic, were neither like those from benzyl-ethyl barbituric acid nor those from benzyl-butyl barbituric acid. The animals first exhibited a period of increasing restlessness and excitement, then a spastic gait, and then showed a few clonic twitches of the neck muscles. A few minutes later these neck clonics were supplanted by a generalized tonic extensor convulsion with occasional quick clonic twitches of the legs becoming manifest. Opisthotonus was usually marked. The animal then rolled over and over. Following this a short period of depression was evident. Such convulsive response is recurrent for hours, thus after 0.5 mM/kg. of dilantin the animals were still convulsing after 3 hours, and after 1.0 mM/kg. two mice were still showing recurrent convulsions after 27 hours. In several of the rabbits following 0.2 mM/kg. of dilantin a more or less marked muscular tenseness became apparent.

Acute toxicities. The acute toxicities of this series of compounds have been determined on white mice kept at constant temperature of 29–30°C. during the period of observation. The statistical data are summarized with the hypnotic activities given in table 5. It will be seen that the series shows no consistent relationship between toxicity and chemical constitution unless it be that compounds having an odd number of carbon atoms in the alkyl chain are less toxic than those with an even number, but the differences are not great. It does appear that the introduction of the benzyl group makes the derivatives more toxic.

In rabbits, the LD-50 for the phenyl-butyl barbituric acid was found to be about 1.5 mM/kg. or about three times that reported by Fitch and Tatum (1932) for phenyl-ethyl barbituric acid (phenobarbital).

DISCUSSION

The convulsions resulting from passage of a 60 cycle alternating current through the brain are typically epileptiform. Löwenbach and Lyman (1940)

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the recurrence of convulsive seizures was more prolonged, for example, 0.5 mM/kg. showed recurrences for 7 hours, while lower doses of 0.4, 0.2, and 0.15 mM/kg. showed recurrences for 4, 3 and 2 hours respectively. This may indicate an inactivation or elimination of the surplus at a fairly uniform rate to a level below the threshold required for action.

FITCH, R. H. AND TATUM, A. L.: J. Pharmacol., 44: 325, 1932.
JESSNER, L. AND RYAN, V. G.: Shock Treatment in Psychiatry. Grune and Stratton, New York, 1941, p. 105.
KNOEFEL, P. K.: J. Pharmacol., 84: 26, 1945.
KNOEFEL, P. K. AND LEHMANN, G.: J. Pharmacol., 76: 194, 1942.
KOZELKA, F. L., HINE, C. H. AND GREIBER, M. F.: Fed. Proc., 1: 156, 1942.
LÖWENBACH, H. AND LYMAN, R. S.: J. Neurol. & Psychiat., 3: 336, 1940.
MERRITT, H. H., PUTNAM, T. J. AND SCHWAB, D. M.: Arch. Neurol. & Psychiat., 39: 1003, 1938.
MERRITT, H. H., PUTNAM, T. J. AND SCHWAB, D. M.: Trans. Am. Neurol. Assn., 63: 123, 1938.
SHONLE, H. A.: J. Ind. & Eng. Chem., 23: 1104, 1931.
SHONLE, H. A. AND MOMENT, A.: J. Am. Chem. Soc., 45: 243, 1923.
SPIEGEL, E. A.: J. Lab. & Clin. Med., 22: 1274, 1937.
SWANSON, E. E.: Proc. Soc. Exper. Biol. & Med., 31: 963, 1934.
TAINTER, M. L., TAINTER, E. G., LAWRENCE, W. S., NEURU, E. N., LACKEY, R. W., LUDUENA, F. P., KIRTLAND, H. B. AND GONZALEZ, R. I.: J. Pharmacol., 79: 42, 1943.

phenyl group decreases rather than increases this activity. With the phenyl-alkyl derivatives the anticonvulsant activity is shown only to 5 carbon atoms in the alkyl group. There is apparently a maximum of this activity in the butyl derivative and a branching of the alkyl group, as in the phenyl-*iso*butyl derivative, lessens the anticonvulsant efficiency to even somewhat less than that of the corresponding propyl compound.

It has been found by Dox and Yoder (1922), and further observed by Shonle and Moment (1923), that the presence of a benzyl group in the 5- position in certain 5-alkyl barbituric acids appears to result in marked convulsant activity. The present investigation bears this out and extends the observations to include the benzyl-butyl derivative. It is of interest to note that the hypnotic activities of the benzyl-ethyl derivative and of the isomeric phenyl-propyl derivative are almost identical. However, with regard to anticonvulsant activities, the phenyl-propyl compound is about 8 times more effective than its convulsion-producing isomer. Correspondingly, benzyl-butyl barbituric acid is about 3 times less active than the isomeric phenyl-amyl compound in anticonvulsant activity.

SUMMARY

1. Additional evidence, from studies of the comparative central actions of dialkyl, phenyl-alkyl and diphenyl barbituric acids, is presented to show that the mechanism which raises the convulsant threshold is different from the mechanism producing hypnosis as evidenced by ataxia and drowsiness.

2. Anticonvulsant activity of barbituric acid derivatives is intensified by the presence of one 5-phenyl group, diminished by the presence of a second 5-phenyl group, and maximal with the phenyl-butyl compound in the series of phenyl-alkyl barbituric acids.

3. Among the 5-phenyl-5-alkyl barbituric acids the widest range between anticonvulsant and hypnotic activities was found to be with 5-phenyl-5-butyl barbituric acid.

4. Of the ten dialkyl and phenyl-alkyl barbituric acids studied, 5-phenyl-5-butyl barbituric acid showed the most marked anticonvulsant activity at dose levels below the hypnotic dose range.

5. The hypnotic activities of the phenyl-alkyl barbituric acids are greatest with the compounds containing the smallest alkyl groups, and this type of central depressant activity decreases with increasing size of the alkyl group.

REFERENCES

ALBERTONI, P.: Arch. f. exper. Path. u. Pharmakol., 15: 248, 1882.

ALLES, G. A., ELLIS, C. H., FEIGEN, G. A. AND REDEMANN, M. A.; Univ. Calif. Publ. Pharmacol., 2: 173, 1946.

BARNES, H. M. AND MCELVAIN, S. M.: J. Am. Chem. Soc., 59: 2348, 1937.

BLISS, C. I.: Quart. J. Pharm. & Pharmacol., 11: 192, 1938.

BUTLER, T. C. AND BUSH, M. T.: Proc. Soc. Exper. Biol. & Med., 50: 232, 1942.

CERLETTI, V. AND BINI, L.: Arch. gen. neurol. psichiat. psichoanal., 19: 266, 1938.

COHEN, B. AND MYERSON, A.: Am. J. Psychiat., 95: 371, 1938.

DOX, A. W. AND YODER, L.: J. Am. Chem. Soc., 44: 1141, 1922.

Tissues are analyzed by the method of Simesen (7). Conjugated derivatives in urine are hydrolyzed by acidification with an equal volume of 4N hydrochloric acid, heating in a boiling water bath for an hour, followed by determination in the usual way. Conjugated derivatives in feces are hydrolyzed by treating the alcoholic extracts with an equal volume of 2N hydrochloric acid for an hour at 100°C.

Acute and chronic toxicity. The toxicity of DBS has been studied chiefly in white mice along with some incidental observations in albino rats and rabbits. The drug was administered orally to mice as a suspension in gum tragacanth solution, and the concentration was so regulated that a dose would be contained in 0.1 to 0.5 cc. Six separate acute toxicity experiments were run. The four which were in the best agreement, involving a total of 257 mice, gave the LD_{50} as 3.2 ± 0.3 gm./kgm.[1] The other two tests, involving 137 mice, gave a value of about 7 gm./kgm. These results served to support the contention of Long, Haviland, and Edwards (9) that the acute toxicity of a poorly soluble sulfonamide compound cannot reliably be determined by oral administration. They state that the method of choice is the subcutaneous administration of the sodium salt. The acute toxicities of sulfanilamide, sulfathiazole, and of DBS were therefore compared in that way. The dosage levels used were 0.75, 1.5, 3.0, and 4.5 gm./kgm. (calculated as the free acid), with 33 mice in each group. The LD_{50} found for both sulfanilamide and sulfathiazole was 1.5 ± 0.3 gm./kgm., and for DBS, 2.5 ± 0.35 gm./kgm. Long, Haviland, and Edwards found the LD_{50} for sodium sulfanilamide to be about 2.8 gm./kgm., and for sodium sulfathiazole 2.0 gm./kgm. (both calculated as the free acid). As for oral subacute toxicity, the highest dose of DBS which mice could tolerate was 1 gm./kgm./day; when repeated daily for 14 days, this amount killed 17 of 31 animals.

Rabbits tolerated 250 mgm./kgm. orally for 3 days without obvious signs of toxicity. When they received 500 mgm./kgm./day for 6 days there were no immediate deaths, but 2 of 5 animals died during the following week and the others survived as long as observed.[2]

Albino rats were somewhat variable in their response to repeated medication with DBS. In one experiment, two oral doses of 1 gm./kgm./day caused such depression that no dose was given on the third day. However, 11 of the 12 animals survived a repetition of the dose on the fourth day.[3] On the other hand, in a subsequent experiment, 24 rats received 750 mgm./kgm. daily for 6 days, followed by 1 gm. per kgm. daily from the eighth to thirteenth days and 2 gm./kgm./daily from the fifteenth to the twenty-fourth day (omitting the twenty-first) with only 6 deaths.

[1] The standard error was estimated graphically by the method of Miller and Tainter (8).

[2] DBS was found in the liver and kidney tissue of these animals, the concentration (without acid hydrolysis) being of the order of 5 mg./100 gm. Histopathological examination of the kidneys showed moderate to severe diffuse nephrosis.

[3] The animals were sacrificed on the fifth day and representative tissues of two were removed for pathological study. In these animals the spleens, hearts, livers and lungs were normal. In the kidneys there was dilatation of the tubules, and these also contained calcified concretions. There was no necrosis in any organ.

3′,5′-DIBROMOSULFANILANILIDE: TOXICITY, ABSORPTION, AND METABOLISM

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Received for publication January 11, 1947

A series of investigations recently reported from this laboratory (1) demonstrated that a group of the sulfanilylanilides is very effective in vitro against a variety of pathogenic microorganisms. Behnisch, Klarer, and Mietzsch (2) first described the preparation of one of the principal compounds of this group, 3′,5′-dibromosulfanilanilide (hereinafter referred to as DBS), which they reported to be effective in the treatment of avian malaria. Marshall, Litchfield, and White (3) found that DBS and its dichlor analogue (DCS) were highly active against both cathemerium and lophurae malaria in ducks. Against the latter infection the two drugs were not significantly antagonized by an amount of p-aminobenzoic acid which completely antagonized sulfadiazine. Goetchius and Lawrence (1) noted that the antibacterial action of these two sulfanilanilides was not opposed by PABA. More recently, however, Schmidt and Sesler (3a) have shown that this generalization must be qualified. Specifically, they found that the action of DBS and DCS against Gram-positive cocci was not affected by PABA, while the action against Gram-negative cocci was uniformly blocked. No favorable effects were noted in vivo when infections with β-hemolytic streptococcus or pneumococcus were treated with DBS or DCS. The observation of Goetchius and Lawrence that DBS is particularly effective against a sulfonamide-resistant strain of N. gonorrhoeae led to the study reported in this paper.

Physical properties of DBS. The method of preparation of DBS and related compounds has been described in detail by Kaplan and Leubner (4). The compound is weakly acidic, a 1% solution of the sodium salt having a pH of 9.8. The solubility of DBS in water is very limited, some typical figures being as follows: about 0.4 mgm. is dissolved by 100 cc. of distilled water at 22°C., and 1.3 mgm. at 37°C. In 2% aqueous sodium bicarbonate at 37°C. the solubility is 9.5 mgm. per 100 cc. In 0.05 M phosphate buffer at pH 8 and 22°C. the solubility is 3.4 mgm. per 100 cc. These values were determined by the usual saturation methods.

Chemical Determination. DBS is readily determined by the method of Bratton and Marshall (5). In order to keep the colored diazo compound in solution, however, it is necessary that the final solution contain 40 volumes % of 95% alcohol; hence, after the treatment with sulfamic acid, 10 cc. of alcohol are added for each 25 cc. final volume, with due allowance being made if any alcohol is already present. For the determination of DBS in blood, the early alcoholic precipitation method of Marshall, Emerson and Cutting (6) has been used, with recoveries of 95–100%. Feces to be analyzed are first dried, then ground and thoroughly mixed. They are extracted three times, with about 50 parts of boiling 95% alcohol, and aliquots of the combined extracts are taken for analysis. After the above treatment the fecal residues contain no material which gives a diazo reaction.

(i.e. capable of diazotization without preliminary hydrolysis) and conjugated DBS. The results are given in table 2.

About half of the total drug given was recovered and less than ⅙ was in the feces, thus indicating good absorption. The urinary concentrations (i.e., "direct") were very high, and far exceeded the known solubility of the drug even at the pH of rabbit urine. This point is considered more in detail below.

Metabolism of DBS in the rabbit. The analytical values obtained indicate that in this species roughly half of the drug appearing in the urine is conjugated. The

TABLE 2

Excretion of DBS by rabbits receiving 250 mgm./kgm. orally daily for 3 days

GROUP	DAY OF EXPT.	TOTAL DOSE, MGM.	FECAL EXCRETION, MGM.		URINARY EXCRETION				TOTAL EXCRETION MGM.
					Mgm.		Mgm. %		
			Dir.‡	Conjug.	Dir.	Conjug.	Dir.	Conjug.	
I	1	2880	101	0	300	225	67	50	626
	2	2880	155	18	541	207	67	26	921
	3	2880	264	0	515	424	79	66	1203
	4	0	92	0	399	117	89	25	608
	5	0	0	0	180	157	42	38	337
	6	0	0	0	163*	92	39	22	225
Total		8640	612	18	2098	1222			3950
II	1	2420	184	0	279	109	53	19	572
	2	2420	290	25	422	187	56	24	924
	3	2420	388	36	545	413	81	64	1382
	4	0	119	0	249	62	57	15	430
	5	0	0	0	115	164	23	38	279
	6	0	0	0	†85	199	29	69	284
Total		7260	981	61	1695	1134			3871

* On 7th day found 86 mgm. additional.
† On 7th day found 32 mgm. additional.
‡ "Direct" DBS is determined on urine without preliminary extraction or hydrolysis.

identity of the conjugated form has not been established but it is known that the combination is very labile since it is completely hydrolyzed simply by heating for an hour at 100°C. without adjustment of pH. The "direct" reacting material in the urine was shown to be free DBS as follows, using the method of Scudi and Silber (11):

The urine was acidified to pH 2.5 with sulfuric acid and after 24 hours refrigeration, the precipitate was collected on a filter. The dried precipitate was dissolved in warm butanol, the insoluble material was filtered off, and the solution was extracted with 0.1 N NaOH. The substance which was extracted could be precipitated by acid, but the amount was very small and it could not be crystallized. It gave a positive diazo reaction and a positive Tollen's test for glycuronic acid.

Absorption and metabolism. The low solubility of DBS precludes rapid and extensive absorption. Nevertheless, it was hoped that definitely bactericidal blood levels could be achieved in spite of these limitations. Absorption experiments were conducted in rats, dogs, rabbits, and men to study this point, with the following results.

Albino rat. Studies of blood levels following the oral administration of 250 mgm./kgm. are given in table 1. They compare favorably with those obtained following similar doses of sulfathiazole (10) and indicate good absorption. In metabolism experiments, it was found that of oral doses of 200 and 1000 mgm./kgm. to rats, only 10 and 16% respectively was found in the feces, which

TABLE 1

Whole blood levels of DBS in the albino rat and the dog following oral administration

Hours after administration	A. Albino rat‡		B. Dog§					
	Dose	Mean free DBS	Dose	Mean DBS, mgm.% ± S.E.		Dose	Mean DBS, mgm.% ± S.E.*	
				Free	Conjug.		Free	Conjug.
	mgm./kgm.	mgm.% ± S.E.*	mgm./kgm.			mgm./kgm.†		
2	250	4.6 ± 0.4	60	4.2 ± 1.5	1.7 ± 1.2	60	1.1 ± 0.6	0.3 ± 0.3
4	250	6.9 ± 0.5	60	2.8 ± 1.1	1.4 ± 0.4	60	2.1 ± 2.4	0.4 ± 0.4
6	250	6.8 ± 0.4	60	3.0 ± 1.1	1.3 ± 1.4	60	0.4 ± 0.5	0
8	250	7.6 ± 0.3	60	2.1 ± 0.2	0	60	0.7 ± 0.9	0.1 ± 0.1
12	250	4.1 ± 0.3						
16	250	5.1 ± 0.6						
20	250	3.8 ± 0.5						
24	250	trace	60	0.2 ± 0.2	0.4 ± 0.4			

* Calculated mathematically (8).
† With 240 mgm. of $NaHCO_3$.
‡ There were 5 to 10 rats in each group.
§ There were 4 dogs to each group, the same animals for each experiment.

also indicates good absorption. However, only small amounts of the drug (mostly conjugated) were found in the urine, and the fate of the remainder was not clear.

Dog. Typical blood levels following the oral administration of 60 mgm./kgm. to this species are also included in table 1. Erratic absorption is indicated, since it was impossible to duplicate results from one experiment to another, and the administration of large doses of bicarbonate failed to improve absorption. Only a minute fraction of the oral doses appeared in the urine.

Rabbit. As judged from the amounts excreted in the urine, the rabbit appears to absorb DBS quite well. The experimental methods are described below.

Two groups of three rabbits were used as subjects. Urine was collected quantitatively and feces were caught on a wire screen. By means of a stomach tube each animal received a dose of 250 mgm./kgm. of DBS, as a 2.5% suspension in gum tragacanth solution, on each of three successive days. Urine and feces were collected for six days following the first dose and were analyzed for "direct"

to dryness. The bottom of the crucible is covered with a ¼ inch layer of fusion mixture (285 parts powdered NaOH, 15 parts KNO_3). The organic matter is carefully destroyed by fusion and enough additional KNO_3 is added to oxidize the carbon particles. After cooling, the melt is dissolved in warm water, made up to 25 cc., and centrifuged. Aliquots are taken for analysis in duplicate. On a third

TABLE 3

Absorption and excretion of DBS by four human subjects following ingestion by each of a 4 gm. dose

SUBJECT	TIME AFTER DOSAGE	BLOOD DBS, MGM. %		URINARY CONTENT OF DBS				
				Milligrams			Mgm. %	
		Free	Total	Interval	Direct	Total	Direct	Total
	hrs.			*hrs.*				
A	1	0	0	0–12	33	306	5.5	57.0
	3	0	0.7					
	5	0.5	3.0	12–24	34	368	6.9	75.3
	8	0.2	4.0					
	24	0	2.9	24–36	61	194	1.3	41.3
B*	1	0	0.6	0–12	38	182	6.2	29.3
	3	0.9	1.7					
	5	1.0	3.3	12–24	84	423	5.4	27.3
	8	1.8	5.8					
	24	—	—	24–36	—	—	—	—
C	1	0	0.4	0–12	8	138	1.3	22.3
	3	0.8	2.0					
	5	0.7	2.3	12–24	14	236	2.4	40.0
	8	0.7	3.0					
	24	0	2.2	24–36	—	—	—	—
D	1	0	1.1	0–12	15	143	4.1	39.6
	3	1.0	1.9					
	5	0.7	1.4	12–24	2	137	0.3	27.3
	8	0	2.0					
	24	0	2.0	24–36	1	161	0.1	25.6

* The slow rate of excretion of DBS is shown by another experiment on Subject B. After the ingestion of 4 gms. of DBS the mgms. of total drug excreted were as follows: 0–24 hrs., 561; 24–48 hrs., 565; 48–72 hrs., 131; 72–96 hrs., 122; and 96–120 hrs., 65. This accounts for 1444 mgm., or 36.1% of the dose.

aliquot the amount of 5N sulfuric acid needed for neutralization is determined. This amount is added to the other aliquots, followed by 5 cc. of 40% NaH_2PO_4 and 10 cc. of commercial Clorox. From this point on the procedure is exactly as described by Friedman: the excess of hypochlorite is destroyed by adding sodium formate, and the bromate is titrated with thiosulfate. The method is very precise and the recovery of bromine added to urine as DBS is ± 2 per cent. It was now applied as described below.

The butanol-soluble material was isolated by evaporation of the butanol in vacuo, solution of the residue in water, and precipitation with acetic acid. The precipitate was readily crystallized from hot 25% ethanol and proved to be chemically pure DBS. The purity was demonstrated by melting point, mixed melting point, and colorimetric analysis.

That it is possible for such an insoluble substance to exist in high concentration in rabbit urine is demonstrated by the following experiment. Two hundred mgm. of DBS were placed in a graduated cylinder and 3 drops of 10% NaOH were added followed by enough water to dissolve the sodium salt (about 12 drops). Rabbit urine was then added to the 100 cc. mark and the mixture was filtered through Whatman No. 1 paper until perfectly clear. The resulting pH was 8.4, which is about normal for rabbit urine, and the concentration of DBS was 140 mgm.%. However, when rabbit urine of the same pH was shaken overnight with an excess of DBS, only 15 mgm.% went into solution. Rabbit urine, therefore, is capable of retaining in solution quantities of DBS which far exceed the apparent aqueous solubility.

Human Subjects. For the purpose of determining blood levels in man following oral ingestion of DBS, the drug was administered to four subjects after a light breakfast, the individual dose being 4 gms. or roughly 60 mgm./kgm. Blood samples were taken at intervals of 1, 3, 5, 8 and 24 hours thereafter, and were analyzed for "direct" and conjugated DBS. Urine samples were collected for 0–12, 12–24, and 24–36 hours after dosage, and they were similarly analyzed. The results are given in table 3.

The dosage of DBS used in this experiment was so chosen that the blood levels could be compared with those obtained by Barlow and Climenko (12) following the administration of sulfapyridine and sulfathiazole. Evidently the absorption and metabolism of DBS follows an entirely different course since it never attains very high levels in the blood as the free drug. On the other hand, it is present in the conjugated form even up to 24 hours and reaches a maximum at 8 hours after administration. The logical interpretation of the data is that the drug is largely removed from the portal blood by the liver, conjugated, and then slowly released for excretion by the kidneys. This interpretation also explains satisfactorily the results of the urinary analyses. Appreciable amounts of free drug have been found only in the first 12 hours after administration, but conjugated DBS appears in the urine in significant amounts up to 120 hours (see table 4). This is in marked contrast to such drugs as sulfanilamide and sulfathiazole, the urinary excretion of which is largely complete within 48 hours (13, 14).

The relatively small amount of DBS recovered from the urine of some human subjects raised the question whether the compound might be excreted in a form not detected by the analytical methods which were being used. The presence of bromine in the molecule provides a convenient check on this point since all bromine absorbed must eventually appear in the urine. A convenient method for the determination of organic bromine in urine proved to be the bromide method of Friedman (15) with some modifications. The procedure follows:

A 10–25 cc. aliquot of urine is measured into a nickel crucible and is evaporated

to dryness. The bottom of the crucible is covered with a ¼ inch layer of fusion mixture (285 parts powdered NaOH, 15 parts KNO_3). The organic matter is carefully destroyed by fusion and enough additional KNO_3 is added to oxidize the carbon particles. After cooling, the melt is dissolved in warm water, made up to 25 cc., and centrifuged. Aliquots are taken for analysis in duplicate. On a third

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	24	—	—	24–36	—	—	—	—
C	1	0	0.4	0–12	8	138	1.3	22.3
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	24	0	2.2	24–36	—	—	—	—
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Four human subjects took doses of 4 gm. of DBS orally, and urine was collected for each of the five following days. The urines were analyzed for total DBS (following acid hydrolysis) and for total bromine, as described above. From the total bromine was subtracted the normal bromide excretion as previously determined on these individuals (by the method of Friedman). The results are given in table 4; they demonstrate that the colorimetric analysis accounts for nearly as much of the drug as does the bromine analysis. While there were differences in the results on occasional samples, it is evident that the colorimetric method of analysis is not failing to detect any significant part of the excreted drug or of its metabolic products.

TABLE 4

Correlation of two methods of analysis of urinary excretion of DBS by 4 human subjects following the ingestion of a 4 gm. dose

SUBJECT	METHODS OF ANALYSIS	PERIOD OF TIME AFTER INGESTION					TOTAL EXCRETION, MGM.
		0–24 hrs.	24–48 hours	48–72 hours	72–96 hours	96–120 hours	
A	Colorimetric*	172	194	94	44	42	546
	Organic Br	175	228	114	72	27	616
B†	Colorimetric	572	473	187	134	65	1431
	Organic Br	592	504	255	139	76	1566
C	Colorimetric	115	192	209	159	118	793
	Organic Br	113	190	225	154	124	806
D	Colorimetric	197	240	124	94	—	655
	Organic Br	196	229	124	88	—	637

* All figures are in terms of mgm. of total drug (determined after acid hydrolysis).
† Same as subject B, table 3.

These data, along with those given in table 3, indicate that there are very appreciable differences in the extent to which human subjects absorb DBS. The average absorption would not appear to exceed 25%.

Nature of the conjugated excretion product. In view of the large proportion of DBS which is excreted in the conjugated form, it was considered desirable to isolate this substance (or substances) and to determine whether it has any antibacterial action. The process used for its isolation and crystallization is described below:

The urine is acidified to pH 2 and extracted twice with an equal volume of ether (by mechanical stirring for an hour each time). The ether is separated off and extracted by stirring for a few minutes with a liter of phosphate buffer containing 50 gms. $Na_2HPO_4 \cdot 12H_2O$, and 15 gms. $NaH_2PO_4 \cdot H_2O$. The ether layer should become colorless. The aqueous layer is now adjusted to pH 2.5 and extracted by shaking with an equal volume of chloroform, the latter being discarded. Extraction with ether is then made several times until no further color is evident in a fresh extract. The ether is dried over sodium sulfate, filtered, and evaporated to

about 200 cc. An excess of benzylamine is added to precipitate the salts of the various acids present. The precipitate is allowed to settle in the refrigerator overnight.

The precipitated salts are now taken up in a small volume of methanol, two volumes of chloroform are added, followed by ether until the mixture becomes just cloudy. When the vessel is set aside in the refrigerator overnight, crystallization will usually occur. If it does not, an oil will separate and the fractionation must be repeated. To do this, the solvents are evaporated off, the residue is taken up in 4% HCl and the organic acids are extracted into ether. The process given above is then repeated exactly beginning with the phosphate extraction, for which about 200 cc. of buffer solution may be used. Correspondingly smaller amounts of materials are used throughout. In this manner a considerable amount of impurities is eliminated and the benzylamine salt will usually crystallize. It characteristically collects (during overnight refrigeration) as small needles on the sides and bottom of the vessel. The needles are intrinsically colorless but are ordinarily somewhat discolored by brown pigment.

To recrystallize, the crystals are dissolved in the least possible volume of methanol; two volumes of chloroform are added, followed by two volumes of ether. After about 10 minutes at room temperature crystallization is complete. The product is collected on a Jena filter, washed with ether, and dried. M.p. when pure, 185–186°C. (dec.). From the mother liquor a further crop of less pure crystals may be obtained by adding more ether.

The compound was subjected to elementary analysis,[4] and hydrolysis to determine DBS. Complete hydrolysis required heating in an autoclave with 4% HCl (15 lb./15 min.).

The analytical data follow:

Analysis, %: C, 42.61; H, 4.35; N, 6.03; S, 4.83; Br, 22.48; DBS, 58.0
Calculated for: $C_{25}H_{31}N_3O_9SBr_2$: C, 42.52; H, 4.40; N, 5.93; S, 4.52; Br, 22.53; DBS, 57.3; Mol. wt. 709.2

When the known constituents, namely benzylamine and DBS, are subtracted, the residue proves to have the empirical formula (after adding water for hydrolysis) $C_6H_{14}O_8$.[5] The formula could also be $C_6H_{12}O_7$ since water added in hydrolysis (as for example, to an aldehyde group) is not always retained.

The carbohydrate nature of the conjugate group is evident from the analysis

[4] The analyses were made in part by the laboratories of Dr. Carl Tiedcke, 366 Fifth Ave., New York City, in part by our own analytical laboratories under the direction of Mr. Morris Auerbach and in part by the author. All determinations were repeated until a close check on each was obtained.

[5] By methods identical with those described in this paper a similar conjugated compound has been isolated from the urine of a subject who ingested 2 gms. of the dichlor analogue of DBS (DCS). The melting point is also 185–186°C. and elementary analysis shows it to have the empirical formula $C_{25}H_{27}N_3SO_9Cl_2$. When the known constituents, namely DCS and benzylamine, are subtracted, the residue has the formula $C_6H_{10}O_8$ or $C_6H_8O_7$. The half-wave potential of the benzylamine salt (determined by Dr. F. C. Nachod) is −1.9 v. at pH 7, identical with that of levulose.

and the same conclusion was indicated by applying the Molisch reaction as modified by Foulger (16). The lavender color obtained, however, was not comparable to that given by hexoses such as glucose (red) and levulose (purple) or by 5-ketogluconic acid (red). The Tollen's naphthoresorcinol test, when run on quantities of 1–2 mgm., gave an atypical reaction (yellow-green fluorescence in the aqueous layer, faint lavender in the ether layer). The Bial's orcinol reaction (17) was also negative, indicating that the carbohydrate is not glycuronic acid.

Other pertinent facts regarding the compound are as follows:

1. Acid hydrolysis. When heated for an hour at 100°C. with HCl the extent of hydrolysis depends on the acid concentration as follows: in 0.02N acid, 7% hydrolyzed; in 0.2N acid, 36% hydrolyzed; in 2.5N acid, 95% hydrolyzed. The hydrolytic reaction was followed colorimetrically. This very slow hydrolytic reaction may modify the response to the color tests mentioned above since they depend on rapid liberation of the carbohydrate by strong mineral acid.

2. Alkaline hydrolysis. In 0.1N NaOH, the hydrolysis was 50% complete in 45 minutes at 100°C. When the resulting solution was acidified to pH 4, a crystalline precipitate formed (a better yield is obtained if N NaOH is used for the hydrolysis). On recrystallization from hot 25% alcohol, it proved to be pure DBS as was demonstrated by melting point, mixed melting point, and nitrogen content.

3. Qualitative tests. The conjugated DBS reduces ammoniacal silver instantly in the cold and reduces Benedict's solution rapidly at 100°C. The latter is reduced slowly at 50°C., but not at 37°C. The substance does not decolorize Tillman's dye, but it does color Schiff's reagent very slowly, the color becoming maximal in 24 hours.

4. Other quantitative data.

a. Reduction of ammoniacal silver. In 24 hours at 37°C. one m.eq. of conjugate reduces 1.8 m.eq. of silver. The reddish ether-soluble product contains 4.5% N, indicating a molecular weight of about 620 and, therefore, that the six-carbon residue is intact.

b. Reduction of hypoiodite. In 25 minutes at room temperature, in dilute Na_2CO_3 solution, one m.eq. of conjugate reduces 4 m.eq. of iodine. The product has a titratable acidic group other than the sulfonamide hydrogen. This product (derived from DCS conjugate) contains 7.74% N, indicating a molecular weight of 363, which with allowance for the parent drug (mol. wt. 317) leaves a side-chain of mol. wt. 46, or not more than 2 carbon atoms.

c. Reduction of periodic acid. In one hour at room temperature one m.eq. of conjugate reduces 10 ± 2 m.eq. of periodate. No formaldehyde is formed in the process, indicating the absence of a primary alcohol group. The product is a chocolate-colored precipitate which is not identical with the parent drug but analysis for N indicates a molecular weight only slightly greater than that of the parent drug. The carbon side-chain is therefore almost completely destroyed by the oxidation.

d. Reduction of alkaline ferricyanide. When determined by the Folin-Malmros blood sugar method, the conjugate contains the equivalent of 23% glucose.

Theory, assuming one available reducing group per mole, 25.5%. Under the conditions of this reaction, no appreciable hydrolysis of the conjugate occurs.

e. Alkali equivalent. Titrating with dilute NaOH and using alizarine yellow (pH 11–12) as indicator, the equivalent weight is 354.

f. Hydroxyl groups. (Determined by reaction with acetic anhydride in pyridine.). The conjugate contains 4 hydroxyl groups, with due allowance being made for the fact that benzylamine also reacts with acetic anhydride, and when this occurs an acidic group becomes available for titration.

g. Amino nitrogen. As determined by the method of Russell (18), the content is 2.0%, originating with the benzylamine.

h. Optical activity. In methanol solution $[\alpha]_{22}^{D} = -57°$.

i. Antibacterial action. Very slight: the compound is bacteriostatic and bactericidal against *Streptococcus* C-203 at 1:2000, and inactive against *Brucella abortus* at 1:1000.[6]

DISCUSSION

The chief point of interest regarding DBS is the manner in which it is conjugated. The attached radical is a carbohydrate having the empirical formula $C_6H_{10-14}O_8$, or $C_6H_{8-12}O_7$. No definite structural formula can be suggested for the compound at this time. However, some of the significant features of its chemical nature which have been established are these:

1. The carbohydrate contains an acidic group capable of combining with benzylamine. The compound formed is neutral, therefore the acidic group is presumably a carboxyl. The ease of dissociation suggests that the compound is a salt.
2. The compound contains 4 hydroxyl groups other than that involved in the carboxyl.
3. The compound shows a strong reducing action even in the cold. In this respect it is intermediate in action between such strongly reducing compounds as 5-ketogluconic acid (19), and the less strongly reducing 2-ketogulonic acid.[7]
4. The carbohydrate is linked to the C_4 amino group only, thus eliminating the direct diazo reaction, and the compound has also no appreciable antibacterial action until hydrolysis has occurred. The C-N linkage is quite stable, being of the same general order of stability as the acid amide linkage (as in acetyl-DBS) rather than that of the glucoside type (20). Nevertheless the analytical data would not be in agreement with the idea that the carbohydrate is a C_6 dicarboxylic acid.
5. The fact that the oxidation products of the compound are invariably colored suggests a cyclic arrangement of atoms, or a tendency to go into such an arrangement.

No previous instance of this type of conjugation of aromatic amino compounds

[6] The studies on this point were conducted by Mr. George Goetchius and Mr. J. W. Klimek.

[7] Generous supplies of these compounds were provided by Dr. W. E. Militzer of the University of Nebraska.

has been reported so far as the author is aware. Recently reported examples of sulfonamide-carbohydrate conjugation involve first the introduction of an hydroxyl group into an aromatic ring, followed by conjugation with glycuronic acid at this position. (21, 22, 23).

SUMMARY

The solubility, toxicity, absorption and metabolism of 3′,5′-dibromosulfanilanilanilide have been studied. The compound is poorly absorbed in most species, particularly man, and therapeutic blood levels have not been achieved except in the rat. The excretion of the drug in man is preceded by a process of conjugation with a carbohydrate radical and since this occurs in the N_4 position, the resulting compound is practically devoid of antibacterial action. The conjugation appears to be of a type not previously reported. The exact structure of the conjugate has not been established.

The author is indebted to Dr. F. C. Goble for the pathological studies reported in this paper, and to Herman Herrlich for considerable technical assistance.

REFERENCES

1. Goetchius, G. R., and Lawrence, C. A., J. Bact., 49: 575, 1945; J. Lab. Clin. Med. 31: 336, 1946.
2. Behnisch, R., Klarer, J., and Mietzsch, F., U. S. Patent No. 2,248,911, July 8, 1941.
3. Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., This Journal, 86: 273, 1946.
3a. Schmidt, L. H., and Sesler, C. L., *ibid*, 87: 313, 1946.
4. Kaplan, H., and Leubner, G., J. Am. Chem. Soc. 67: 1076, 1945.
5. Bratton, A. C., and Marshall, E. K., Jr., J. Biol. Chem., 128: 537, 1939.
6. Marshall, E. K., Jr., Emerson, J., Jr., and Cutting, W. C., J. A. M. A. 108: 953, 1937.
7. Simesen, M. H., Arch. exptl. Path. u. Pharmakol., 197: 87, 1940.
8. Miller, L. C., and Tainter, M. L., Proc. Soc. Exptl. Biol. & Med. 57: 261, 1944.
9. Long, P. H., Haviland, J. W., and Edwards, L. B., *ibid*, 43: 328, 1940.
10. Laboratory data.
11. Scudi, J. V. and Silber, R. H., J. Biol. Chem., 156: 343, 1944.
12. Barlow, O. W., and Climenko, D. R., J. A. M. A. 116: 282, 1941.
13. Stewart, J. D., Rourke, G. M., and Allen, J. G., *ibid*, 110: 1885, 1938.
14. Sadusk, J. F., Jr., Blake, F. G., and Seymour, A., Yale Jour. Biol. and Med., 12: 681 1940.
15. Friedman, M. M., J. Biol. Chem., 144: 519, 1942.
16. Foulger, J. H., *ibid*, 92: 345, 1931.
17. Militzer, W. E., Arch. Biochem., 9: 85, 1946.
18. Russell, J. A., J. Biol. Chem., 156: 467, 1944.
19. Militzer, W. E., *ibid*, 154: 325, 1944.
20. Jackson, E. L., J. Am. Chem. Soc. 68: 1438, 1946.
21. Scudi, J. V., Proc. Soc. Exptl. Biol. and Med. 55: 197, 1944.
22. Weber, C. J., Lalich, J. J., and Major, R. H., *ibid*, 53: 190, 1943.
23. Bray, H. G., Neale, F. C., and Thorpe, W. V., Biochem. J. 40: 406, 1946.

THE EFFECT OF THOROTRAST ON ANAPHYLACTIC SHOCK IN THE DOG[1]

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Received for publication January 22, 1947

As a result of numerous investigations, summarized by Dragstedt (1), anaphylactic shock in the dog is generally conceded to result from the liberation of pharmacologically active substances following the injection of an antigen into a sensitized animal. The site at which this fundamental reaction takes place is not definitely known.

The possible participation of the reticulo-endothelial system in the anaphylactic reaction has been considered by several investigators who studied the rôle of this system by attempting to block it prior to the shocking injection. Petersen and coworkers (2) have found that the injection of saccharated iron oxide prevented certain manifestations of anaphylactic shock as judged by the composition of thoracic duct lymph. Only five experimental and two control dogs were used in this study. Mills and Dragstedt (3) were unable to decrease the severity of anaphylactic shock in a large series of dogs by injections of India ink or iron oxide.

The purpose of the present study was to ascertain the effect of thorotrast[3], a more effective blocking agent that previous investigators have used, on anaphylactic shock in the dog. It is well-known that different blocking agents may vary considerably in their ability to inhibit the various functions of the reticulo-endothelial system. Maher (4) has shown that whereas India ink fails to prevent the conjugation of sulfanilamide in the rabbit, thorotrast does so.

EXPERIMENTAL. The protective action of thorotrast in anaphylactic shock in the dog—Adult mongrel dogs were sensitized by two subcutaneous injections of horse serum, 0.3 cc. per kgm. of body weight, given 2 to 3 days apart. Sixteen to 24 days following the first sensitizing injection the dogs were anesthetized with intravenous sodium pentobarbital and received an intravenous injection of horse serum, 0.5 cc. per kgm. of body weight. Blood pressure was recorded by means of a mercury manometer, and clotting time was measured by the Lee and White (5) method before and after the intravenous injection of horse serum.

Fourteen dogs were used in this experiment. Nine received thorotrast in amounts varying from 2 to 6 cc. per kgm. of body weight. The time interval between the injection of thorotrast and the injection of serum was 4 hours in some dogs and 10 minutes in others. Five dogs which received no thorotrast served as controls.

As indicated in table 1, thorotrast had a considerable effect in preventing the fall of blood pressure and the increase in clotting time. The dosage determined

[1] This work was aided by a grant from the First Texas Chemical Company.

[2] Fellow in the Department of Internal Medicine.

[3] Thorotrast is a stabilized colloidal thorium dioxide contrast medium containing 24 to 26 per cent ThO_2 by volume, obtained from the Heyden Chemical Corporation, New York, to whom we are indebted for part of the material used in this study.

has been reported so far as the author is aware. Recently reported examples of sulfonamide-carbohydrate conjugation involve first the introduction of an hydroxyl group into an aromatic ring, followed by conjugation with glycuronic acid at this position. (21, 22, 23).

SUMMARY

The solubility, toxicity, absorption and metabolism of 3′,5′-dibromosulfanilanilanilide have been studied. The compound is poorly absorbed in most species, particularly man, and therapeutic blood levels have not been achieved except in the rat. The excretion of the drug in man is preceded by a process of conjugation with a carbohydrate radical and since this occurs in the N_4 position, the resulting compound is practically devoid of antibacterial action. The conjugation appears to be of a type not previously reported. The exact structure of the conjugate has not been established.

The author is indebted to Dr. F. C. Goble for the pathological studies reported in this paper, and to Herman Herrlich for considerable technical assistance.

REFERENCES

1. Goetchius, G. R., and Lawrence, C. A., J. Bact., 49: 575, 1945; J. Lab. Clin. Med. 31: 336, 1946.
2. Behnisch, R., Klarer, J., and Mietzsch, F., U. S. Patent No. 2,248,911, July 8, 1941.
3. Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., This Journal, 86: 273, 1946.
3a. Schmidt, L. H., and Sesler, C. L., *ibid*, 87: 313, 1946.
4. Kaplan, H., and Leubner, G., J. Am. Chem. Soc. 67: 1076, 1945.
5. Bratton, A. C., and Marshall, E. K., Jr., J. Biol. Chem., 128: 537, 1939.
6. Marshall, E. K., Jr., Emerson, J., Jr., and Cutting, W. C., J. A. M. A. 108: 953, 1937.
7. Simesen, M. H., Arch. exptl. Path. u. Pharmakol., 197: 87, 1940.
8. Miller, L. C., and Tainter, M. L., Proc. Soc. Exptl. Biol. & Med. 57: 261, 1944.
9. Long, P. H., Haviland, J. W., and Edwards, L. B., *ibid*, 43: 328, 1940.
10. Laboratory data.
11. Scudi, J. V. and Silber, R. H., J. Biol. Chem., 156: 343, 1944.
12. Barlow, O. W., and Climenko, D. R., J. A. M. A. 116: 282, 1941.
13. Stewart, J. D., Rourke, G. M., and Allen, J. G., *ibid*, 110: 1885, 1938.
14. Sadusk, J. F., Jr., Blake, F. G., and Seymour, A., Yale Jour. Biol. and Med., 12: 681 1940.
15. Friedman, M. M., J. Biol. Chem., 144: 519, 1942.
16. Foulger, J. H., *ibid*, 92: 345, 1931.
17. Militzer, W. E., Arch. Biochem., 9: 85, 1946.
18. Russell, J. A., J. Biol. Chem., 156: 467, 1944.
19. Militzer, W. E., *ibid*, 154: 325, 1944.
20. Jackson, E. L., J. Am. Chem. Soc. 68: 1438, 1946.
21. Scudi, J. V., Proc. Soc. Exptl. Biol. and Med. 55: 197, 1944.
22. Weber, C. J., Lalich, J. J., and Major, R. H., *ibid*, 53: 190, 1943.
23. Bray, H. G., Neale, F. C., and Thorpe, W. V., Biochem. J. 40: 406, 1946.

Heparin was added to samples of dog's blood in amounts of 0.2 mgm. and 0.4 mgm. per 100 cc. Thorotrast was then added to 0.5 cc. portions of the heparinized blood samples in amounts of 0.01 cc., 0.05 cc., and 0.1 cc. All samples, including the controls, were incubated at 37°C. There was no evidence of clotting in any of the tubes in 4 hours. Thorotrast thus fails to counteract the effect of heparin on dog's blood in vitro.

DISCUSSION

The mechanism of action of thorotrast in the prevention of various manifestations of anaphylactic shock in the dog is of importance because it may elucidate certain aspects of the problem of anaphylaxis which are not well understood. Since thorotrast does not counteract the effects of histamine and heparin, it must act presumably (a) by preventing the antigen from reaching the antibody or (b) by blocking the fundamental reaction which leads to the liberation of histamine and heparin in anaphylactic shock.

Since it is well known that thorotrast tends to be concentrated in the reticulo-endothelial system, the results of the present study suggest that the fundamental reaction in anaphylactic shock in the dog takes place in elements of the reticulo-endothelial system. The apparently contradictory results of Mills and Dragstedt (3) may be explained by the fact that these authors used Indian ink and iron oxide, which are not as efficient blocking agents as is thorotrast. Maher (4) demonstrated the superiority of thorotrast over other blocking agents.

The results of the present study are in harmony with the findings first described by Manwaring (7), which indicate that the liver plays an important rôle in canine anaphylactic shock since elements of the reticulo-endothelial system occur abundantly in the liver. However, since such elements are also widely distributed elsewhere in the body, it is not surprising that Waters and coworkers (8) and others were able to produce anaphylactic shock in the hepatectomized dog.

CONCLUSION

Thorotrast in large doses protects dogs against anaphylactic shock. Since thorotrast has no antihistamine or antiheparin actions, it is suggested that it prevents the liberation of these substances.

REFERENCES

1. Dragstedt, C. A.: Physiol. Rev., 21: 563, 1941.
2. Petersen, W. F., Jaffe, R. H., Levinson, S. A., and Hughes, T. P.: J. Immunol., 8: 367, 1923.
3. Mills, M. A., and Dragstedt, C. A.: J. Immunol., 31: 1, 1936.
4. Maher, F. T.: The Reticulo-Endothelial System in Sulfonamide Activity, The University of Illinois Press, Urbana, 1944.
5. Lee, R. I. and White, P. D.: Am. J. M. Sc. 145: 495, 1913.
6. Jaques, L. B. and Waters, E. T.: J. Physiol., 99: 454, 1941.
7. Manwaring, W. H.: Ztschr. f. Immunitatsforsch. u. exper. Therap., 8: 1, 1911.
8. Waters, E. T., Markowitz, J., and Jaques, L. B.: Science, 87: 582, 1938.

the degree of protection observed. Whereas 2 cc. of thorotrast solution per kgm. of body weight had no protective action, 4 to 6 cc. produced partial or complete protection against anaphylactic shock.

In none of the dogs receiving 4 cc. or more of thorotrast per kgm. of body weight was the clotting time elevated appreciably following the injection of serum, and only one dog in this group showed a significant fall of blood pressure (dog No. 8).

Thorotrast when given alone tended to prolong the clotting time within 4 hours and caused a bleeding tendency in the majority of the animals. Maher (4) observed similar effects in the rabbit.

TABLE 1

The effect of thorotrast on the blood pressure and clotting time in anaphylactic shock induced by horse serum in the dog

DOG NO.	AMOUNT OF THOROTRAST USED IN PRETREATMENT	TIME INTERVAL BETWEEN PRETREATMENT AND THE INJECTION SERUM	OBSERVED DECLINE IN BLOOD PRESSURE	CLOTTING TIME		
				5 Min. prior to injection of serum	10 min. after injection of serum	30 Min. after injection of serum
	cc. per kgm.	*minutes*	*mm. Hg.*	*Minutes*		
1	0		114	8	>360	120
2	0		126	16	>360	>360
3	0		*	8		
4	0		82	8	11	
5	0		89	7	>360	>360
6	2	10	*	4		
7	2	240	75	15	24	180
8	4	240	70	20	23	30
9	4	240	0	9	15	15
10	6	10	0	4	8	9
11	6	10	37	7	15	19
12	6	240	0	23	24	23
13	6	240	0	17	20	16
14	6	240	0	11	19	21

* Died in shock.

The effect of thorotrast on the reaction to histamine—Since the fall of blood pressure in canine anaphylactic shock is generally attributed to the liberation of histamine, it was important to determine whether thorotrast could abolish the effects of histamine. Two dogs (Nos. 7 and 14), which received an injection of 2 cc. and 6 cc. per kgm. respectively of thorotrast solution, were given an intravenous injection of 0.05 mgm. per kgm. of histamine approximately 45 minutes after the injection of horse serum. The blood pressure fell markedly in both dogs. Thorotrast, consequently, does not prevent the effect of histamine on the blood pressure.

The effect of thorotrast on the action of heparin in vitro—The increased clotting time in canine anaphylactic shock is caused by the liberation of heparin (6). Since thorotrast prevented the occurrence of incoagulability of the blood in vivo, its effects on heparin in vitro were investigated.

ratio 77) than they report in cats (epinephrine ratio 184), in agreement with their findings the racemic salt appears to have more pressor activity than would result from the additive action of the two isomers. These investigators have suggested that this is "evidence of a sensitization occurring in the racemic mixture."

(b) *Effect on the Central Nervous System:* The determination of the stimulating effect of these compounds on the central nervous system was made by Frick and Becker of the Sterling-Winthrop Research Institute, according to the method of Schulte, Tainter and Dille (6). They have been kind enough to furnish us the data shown in table 2.

Results obtained indicate that d-N-methyl-β-cyclohexylisopropylamine HCl has much less stimulating effect than does its phenyl analogue. Comparatively large doses (20.0 mgm./kgm.) of the former caused only a small amount of stimulation. Data obtained with l-N-methyl-β-cyclohexylisopropylamine HCl has shown this substance to be distinctly more stimulating than its d-isomer, one-half the dose of the former causing four times more random activity than the latter. Novelli and Tainter (7) haveshown d-N-methyl-β-phenylisopropylamine HCl to have much more excitatory effect on the central nervous system of rats than does its l-isomer. Schulte et al. (6) have reported the l-isomer of ephedrine to be more stimulating than the d-isomer. In this effect, N-methyl-β-cyclohexylisopropylamine more nearly resembles ephedrine.

(c) *Toxicity:* Acute toxicity was determined in albino mice. All test animals were obtained from our own colony, were injected and kept under observation in the colony room where the environmental temperature was maintained at 76°F. The hydrochloride salts of the compounds were dissolved in distilled water and injected intraperitoneally. Injected mice were caged in groups of five and deaths were recorded for seventy-two hours. Racemic N-methyl-β-cyclohexylisopropyllamine HCl and its d- and l-isomers were found to have an LD_{50} of approximately 70 mgm./kgm. It would seem that the toxicity, as determined in these tests, is not influenced by the observed differences in pharmacologic activity. The toxicity of N-methyl-β-phenylisopropylamine HCl equals that of the above substances. Hydrogenation of the phenyl ring does not alter the toxicity of this compound.

Warren and Werner (8a) have reported the toxicity of Neo-Synephrine HCl to be 22.0 ± 4.3 mgm./kgm. when injected subcutaneously in mice. This toxicity value would not be expected from the values we have obtained by intraperitoneal injection. We have found the LD_{50} to be 140 mgm./kgm. Racemic Neo-Synephrine HCl is much less toxic than the l-isomer (table 1). This suggests that the d-isomer has a low toxicity.

Recent articles describing toxicity determinations have stressed the importance of environmental temperature, species of animal used, animal weight (Warren and Werner, 8), and the effect of the number of drug-tested animals per cage (Chance, 9) on the observed mortality. Differences in the reported toxicities are particularly large for the β-phenylisopropylamines. Thus, Warren and Werner (8b) report that the subcutaneous LD_{50} for amphetamine in rats is

THE PHARMACOLOGIC ACTIVITY OF N-METHYL-β-CYCLOHEXYL-ISOPROPYLAMINE HYDROCHLORIDE

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Received for publication January 31, 1947

The pharmacologic activity of various derivatives of β-cyclohexylethyl- and γ-cyclohexylpropyl-amine has been described by Lands, Lewis, and Nash (1). Also, Gunn and Gurd (2) have described the pharmacology of α- and β-cyclohexylethyl- and β-cyclohexylisopropylamine. Zenitz, Macks, and Moore (3) have recently synthesized N-methyl-β-cyclohexylisopropylamine, and this substance with its optically active isomers has been made available to this Laboratory for pharmacologic investigation. The pressor activity in pithed dogs of the optical isomers of the phenyl analogue (desoxyephedrine) has been described by Swanson, Scott, Lee and Chen (4). Inasmuch as we have used dogs with intact nervous systems for our investigation, we have included for comparison data obtained by us with desoxyephedrine and its isomers. Results obtained with the above substances are compared with those obtained with N-methyl-β-(m-hydroxyphenyl)-ethanolamine hydrochloride (Neo-Synephrine HCl) and its optical isomers.

RESULTS: (a) *Effect on Blood Pressure:* Dogs were anesthetized with sodium pentobarbital and prepared for the kymographic recording of carotid blood pressure. All injections were made directly into the exposed femoral vein. Inasmuch as tachyphylaxis is quite marked with both β-phenyl- and β-cyclohexylisopropylamines, only one observation was made for each dog used. Results obtained are shown in table 1. It will be noted that dl-N-methyl-β-cyclohexylisopropylamine HCl has about two-thirds of the activity of its phenyl analogue.

The l-isomer is distinctly more pressor than the d-isomer. This is in sharp contrast to the results obtained with the phenyl analogues, since l-desoxyephedrine is approximately half as active as the d-isomer. Swanson et al. (4) found the l-isomer of desoxyephedrine to be 1.4 times more active than the d-isomer. The difference in the results obtained may result from the difference in the test preparations used, that is, the difference between the response of the pithed dog as compared with one in which the nervous system is intact. That the effect on the central nervous system is not unimportant is suggested by the results shown in table 2.

Racemic Neo-Synephrine HCl is about twenty-five times more pressor than N-methyl-β-cyclohexylisopropylamine HCl. The l-isomer of Neo-Synephrine was found to be 10.5 times more pressor than the d-isomer. Tainter and Stockton (5) have reported the racemic salt to be more active than would have been expected from the comparative activities of the d- and l-isomers. Although we have found the d-isomer to be about 2.3 times more active in dogs (epinephrine

39.0 ±2.3 mgm./kgm. in heavy animals and 165.0 ±16.5 mgm./kgm. in light animals. Chance (9) found the subcutaneous LD_{50} in mice injected with amphetamine to be 117.3 mgm./kgm. when the treated animals were caged individually, but was 14.0 mgm./kgm. when ten treated animals were caged together. This investigator further reports the LD_{50} for d-N-methyl-β-phenylisopropylamine HCl to be 23.26 mgm./kgm. when mice are caged individually, and 7.56 mgm./kgm. when caged in groups of ten. Novelli and Tainter (7), using rats, have reported the subcutaneous toxicity of dl-N-methyl-β-phenylisopropylamine HCl to be greater than that of either the d- or l-isomer, the l-isomer being least toxic. In view of the above data it is difficult to evaluate toxicity reported in the literature. The environmental conditions prevailing in the various laboratories and the varied test procedures used appear to be determining factors in the results obtained.

SUMMARY

1. Racemic N-methyl-β-cyclohexylisopropylamine HCl has about two-thirds of the pressor activity of its phenyl analogue (racemic desoxyephedrine HCl).
2. l-N-Methyl-β-cyclohexylisopropylamine HCl is more pressor and more excitatory on the central nervous system than is the d-isomer.
3. d-N-Methyl-β-phenylisopropylamine HCl is more pressor and more excitatory on the central nervous system than is the l-isomer.
4. All the isomers of N-methyl-β-cyclohexylisopropylamine HCl and N-methyl-β-phenylisopropylamine HCl were found to have the same acute toxicity in mice.
5. Racemic Neo-Synephrine HCl is approximately twenty-five times more pressor than racemic N-methyl-β-cyclohexylisopropylamine HCl, approximately seventeen times more pressor than N-methyl-β-phenylisopropylamine HCl and is approximately one-sixth as toxic.

REFERENCES

1. Lands, A. M., Lewis, J. R., and Nash, V. L.: This Journal, 83: 253, 1945.
2. Gunn, J. A., and Gurd, M. R.: J. Physiol., 97: 453, 1940.
3. Zenitz, B. L., Macks, E. B., and Moore, M. L.: J. Am. Chem. Soc., In Press.
4. Swanson, E. E., Scott, C. C., Lee, H. M., and Chen, K. K.: This Journal, 78: 329, 1943.
5. Tainter, M. L., and Stockton, A. B.: Am. J. Med. Sc., 185: 832, 1933.
6. Schulte, J. W., Tainter, M. L., and Dille, J. M.: Proc. Soc. Exper. Biol. and Med., 42: 242, 1939.
7. Novelli, A. N., and Tainter, M. L.: This Journal 77: 324, 1943.
8. Warren, M. R., and Werner, H. W.: This Journal, (a) 86: 284, 1946. (b) 86: 280, 1946.
9. Chance, M. R. A.: This Journal, 87: 214, 1946.

TABLE 1

The pressor activity in dogs and the toxicity in mice of the optical isomers of N-methyl-β-cyclohexylisopropylamine hydrochloride and related compounds

COMPOUND	NO. OF DOGS USED	DOSE	RISE IN BLOOD PRESSURE	DURATION	APPROX. EPINEPHRINE RATIO*	TOXICITY (intraperitoneal-albino mice) No. of mice used	Approx. LD_{50}
		mgm./kgm.	mm. Hg.	min.			mgm./kgm.
N-Methyl-β-cyclohexylisopropylamine HCl							
d-isomer	5	0.50	31	10->30	400	20	70
dl-mixture	3	0.50	43	10->30	290	18	70
l-isomer	6	0.26	41	8->30	160	20	70
N-Methyl-β-phenylisopropylamine HCl							
d-isomer	6	0.25	54	26->30	116	10	70
dl-mixture	6	0.66	82	40- 64	200	12	70
l-isomer	5	0.50	46	8->30	272	14	70
Neo-Synephrine HCl **							
d-isomer	7	0.145	47	7-11	77		
dl-mixture	13	0.025	53	7-11	12	50	420
l-isomer	15	0.014	47	7-11	7	100	140†
l-Epinephrine	16	0.002	50	2- 3	1	32	4

* Drug equivalent to 1 mgm. of l-epinephrine

† The distribution of deaths does not indicate a definite LD_{50}. The LD_0 was <50 mgm./kgm.; the LD_{100} was >160 mgm./kgm.

** Neo-Synephrine HCl is the l-isomer, used here to indicate substance without reference to rotation.

TABLE 2

Central nervous system stimulation

(Method of Schulte, Tainter and Dille)

COMPOUND	NUMBER OF RATS USED	DOSE*	ACTIVITY: Number of revolutions per hour 1	2	3	4	TOTAL
		mgm./kgm.					
N-Methyl-β-cyclohexylisopropylamine HCl							
d-isomer	6	20.0	15	6	2	5	28
dl-mixture	6	5.0	3	4	3	2	12
dl-mixture	6	30.0	25	6	5	5	41
l-isomer	12	5.0	39	28	9	5	81
l-isomer	6	10.0	33	40	17	9	99
N-Methyl-β-phenylisopropylamine HCl							
d-isomer	12	1.0	61	32	10	5	108
d-isomer	12	2.5	85	51	29	10	175
Control	64	None	6.5	3.2	3.1	3.3	16.1

* The salt in aqueous solution was injected subcutaneously.

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